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PROFESSOR BIRESCHANDRA GUPTA  
(1934—1962)

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## FOREWORD

During the inauguration of the Guha Institute of Biochemistry on August 12, 1972, at the Department of Biochemistry, Calcutta University, it was decided to publish a commemoration volume as a mark of respect to late Professor B. C. Guha, the internationally reputed nutritional biochemist, who did much towards the development of Biochemistry and Nutritional Science in India. Not only as a scientist but also as an organizer of science and technology in India, Professor Guha gave ample evidence of his outstanding personality and leadership during his life time.

A number of scientists from India and abroad responded to our appeal and contributed highly interesting scientific articles of the different aspects of modern biochemistry for the commemoration volume. Besides a few research papers presented by eminent nutritionists of India at the all-India Symposium on "Advancing Frontiers of Nutritional Biochemistry" organised on the occasion of the inauguration of the Guha Institute of Biochemistry, are also included in this volume. All these authors are either personal friends or students of late Professor Guha.

We take this opportunity to express our sincere thanks to the teachers, research workers, staff and students of the Department for their untiring efforts and support to make the occasion a success. We are also grateful to some of the firms : M/s. Bengal Immunity Co. Ltd., M/s. B. C. Chatterjee & Bros., M/s. S. K. Biswas & Co., M/s. Satyacharan Ghosh and others for their generous financial help towards meeting the expenses for this great occasion.

We are indeed very much indebted to Prof. D. S. Kothari, the then Chairman, University Grants Commission, who accompanied by his Development officer, Dr. S. K. Dasgupta flew to Calcutta to inaugurate the Guha Institute of Biochemistry under extremely bad weather. Also we like to express our gratitude to Prof. S. N. Sen, Vice-Chancellor, Calcutta University, who in the midst of his heavy responsibilities, kindly consented to preside over the inauguration ceremony. Our thanks are also due to Prof. P. K. Bose, Pro-Vice-Chancellor (Academic Affairs), Calcutta University, for his active interest and advice towards the success of the function. Lastly, we should like to remember gratefully Dr. P. K. Bose, Dr. B. Mukerji, Prof. M. M. Chakrabarti, Mr. S. Kanjilal, Superintendent, Calcutta University Press, for their advice and co-operation.

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## PROFESSOR BIRES CHANDRA GUHA (1904-1962)

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An essential element in the development of a person is his family background, social environment, early education and inspiration. Bireschandra Guha comes of a family, particularly from his maternal side, which to a great extent, symbolized the aims, ideals and resurgent spirit of the contemporary life of Bengal, if not of India. His maternal uncle late Aswinikumar Datta was a staunch nationalist, a patriot of lofty ideals and character and was one of the architects of our national struggle for independence. Born amidst such a family tradition and atmosphere Guha imbibed, as he grew up, many of the characteristics of his illustrious uncle and his time. The efflorescence of cultural life and aspiration for political emancipation which followed in the wake of renaissance of Bengal in the earlier century, also contributed to the early formative period of his life. In fact these influences had an in-built effect in giving direction and depth to the scientific life of Guha in later days.

Guha had a uniformly brilliant academic career as a student of science. He was naturally drawn to Acharya Prafullachandra whose austere and dedicated life to the cause of science also influenced and inspired him greatly. The time when Guha joined the Science College, Calcutta University, may be considered in many respects as a period of efflorescence for the scientific research in India. Raman, Saha, Bose, Ghosh and many others were the illustrious products of the period. They have not only earned permanent names for themselves amongst the scientists of the world, but also extended the frontiers of knowledge considerably. Parallel with this scientific development other branches of knowledge, literature, philosophy, history, etc. were in the height of scholastic achievements. In fact, the University of Calcutta has by that time become a real "centre of excellence and advancement of knowledge."

Young Guha was fortunate enough to join the post-graduate department of the University College of Science in the creative period of the University. To add to that, there were inspiring teachers, like Acharya Prafullachandra, Jagadishchandra and a galaxy of outstanding scholars who adorned the various departments of the University. All these factors had also profound impact on Guha for determining the future course of his life.

Krebs rightly says, "distinction breeds distinction or in other words distinction develops if nurtured by distinction." This can be most aptly said



about young Guha. He was practically nurtured by Acharya Prafullachandra, who was in the true sense of the word, his spiritual father. After a brief period of post M.Sc. research in the University College of Science, Guha left for England on a Tata Endowment Fellowship for higher studies and research. He was so very bold in the choice of the subject for his future scientific career. He was an organic chemist by training and discipline. At that time biochemistry was not much known in India except in the field of nutrition and allied areas. Prospect of employment on return to India was highly restrictive if not remote. So it required certain boldness of spirit and self confidence on the part of Guha to take to a path of uncertainty and adventure. He joined the laboratory of Professor J. C. Drummond at the Imperial College of Science and Technology, London, and got his Ph.D. there. He then shifted to Cambridge to work in the laboratory of Professor F. G. Hopkins, which was at that time considered as one of the world's leading centres for advanced study and research in biochemistry. Outstanding achievements of Hopkins and his group had attracted many young brilliant workers there from all over the world. Amongst them were Albert Szent-Györgyi, Hans Krebs to mention only a few, who were later awarded Nobel Prize and are at present regarded as architects of modern biochemistry. Krebs himself admits that "scientists are not so much born as made by those who teach them research." Guha had the unique advantage of having initial training with Acharya Prafullachandra, the father of modern chemistry in India and later with Hopkins the spiritual father of modern biochemistry. This is perhaps one of the reasons why young Guha shaped up so brilliantly as a scientist in later life. Cambridge was then at the height of scientific achievements and enjoyed the leadership of the scientific world, particularly in atomic physics. Academic atmosphere of Cambridge was quite unique and exciting. Newer ideas and concepts which later revolutionized the whole field of biological sciences were taking some definite shape. These developments in biological sciences and other interdisciplinary fields also shaped the scientific pattern and perspective of Guha as a true scholar and investigator.

Guha had a very liberal training in Cambridge in the Hopkins laboratory. In describing the then atmosphere that prevailed in the laboratory, Krebs writes, "It was in Hopkin's laboratory where I saw for the first time at close quarters some of the characteristics of what is sometimes referred to as "British ways of life." The Cambridge laboratory included people of many different dispositions, convictions and activities. I saw them argue without quarrelling, quarrel without suspecting, suspect without abusing and criticize without vilifying or ridiculing and praise without flattering."

Love for his country was in the very blood of Guha. The liberal and democratic atmosphere of Cambridge nurtured it into a more concrete and mature form with definite polarization for certain political philosophy. By this time he got his D.Sc. and was now ready to leave for India. Guha's life has been comparatively smooth sailing, marked by uniform success and marred by no untoward events. This sort of successful life has also its compensatory side. Life untouched by any adverse condition or circumstances fails to develop certain toughness of mind and resilience of spirit, which alone immunize a person against disappointment and frustration and make him sturdy enough to pursue his ideals against great setbacks. When Guha returned to India he carried with him a little of Cambridge and its atmosphere of "contagious enthusiasm, broadmindedness and imagination" and above all a deep devotion to science and scientific pursuits. He was full of high hopes, and aspiration. He thought that his scientific achievements and acquisitions will help him to get a suitable place for further development of his capabilities and talents. He however, got a rude shock when he failed to get a professorship in biochemistry in a research institute in Calcutta, simply because of his past political association, which the then alien Government looked upon with considerable askance. He then joined an industrial concern where scope for biochemical research and teaching was practically absent. He soon left that job and joined a college where teaching was at the graduate level without any research facility. All these had a very gnawing effect on his dynamic and superabundant spirit. Because Guha had no hard mental schooling, it was probably very difficulty for him to absorb these disappointments with fortitude and patience. He later got the Professorship of the Department of Applied Chemistry in Calcutta University but his superb training and experience in the field of biochemistry was out of tune with the main objective and teaching programme of the Department. There was practically no laboratory worthy of name for biochemical research in the Department and biochemistry was at that time not even a full subject for postgraduate study in Calcutta University. So Guha was extremely handicapped so far his research was concerned. He lacked requisite fund and a good laboratory for his work. These things, however, do not come without long and hard work. Though Guha might have heard very often from Hopkins that a gold coin can be got in a wayside per chance, but it requires systematic and arduous mining to keep the currency going on. While he was working under such unfavourable conditions, his contemporaries in Cambridge were making history in the field of biochemistry. All these factors in their totality had a profound impact on the inner workings of his sensitive mind. He had so long taken science as his part and mission of life but not as profession of life.

Various setbacks helped to develop slowly an inner dichotomy in him. Guha had considerable mental conflicts before he took to other professions, though in the heart of heart he was for science and scientific pursuits.

For nearly ten years he filled in a number of positions not very much related to biochemistry. There also he showed his outstanding abilities and leadership. In spite of his tremendous success in the different professions and positions, he was the most unhappy man because of his divorce from the real scientific field. The present writer had many intimate occasions to have glimpses of his inner mind during those periods.

Guha was also very fortunate in having for his life's consort an accomplished and illustrious lady like Dr. Phulrenu Guha. In fact she complemented him in every respect.

When his life was full with experience and knowledge garnered from the various walks and ways of life, he perhaps discovered or rather re-discovered, like Goethe's *Faust* that the greatest value in life, that which gives real meaning and the deepest satisfaction is creative achievement. Perhaps with this realization he returned to science fully dedicated to its cause. He now devoted his entire time, energy and his rare leadership and dynamicism in research which soon bore fruits in some spectacular achievements. He was straining too much, as if atoning for his past neglect of science. He was in the height of his research activity, when the end came quite suddenly and unexpectedly. He was, as if, consumed by the very fire which sustained him so long.

Many facets of his myriad mind and multiple positions he held and served with remarkable ability, have been delineated by people who know him more closely and intimately. But to the present writer he is an outstanding scientist, an inspiring teacher and above all a Man and a Soul now redeemed in Self-Dedication.

Let us also not forget, "All our yesterdays have lighted men the way to the scientific creativity of today."

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# CONTROL OF THE REDOX STATE OF THE NICOTINAMIDE—ADENINE DINUCLEOTIDE COUPLE IN RAT LIVER CYTOPLASM

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1. A study has been made of the ability of rat liver *in vivo* to maintain equilibrium in the combined glyceraldehyde 3-phosphate dehydrogenase, 3-phosphoglycerate kinase and lactate dehydrogenase reactions, *i.e.* in the system :

$$\frac{[\text{ATP}]}{[\text{ADP}][\text{HPO}_4^{2-}]} = \frac{[\text{pyruvate}]}{[\text{lactate}]} \cdot \frac{[\text{glyceraldehyde 3-phosphate}]}{[\text{3-phosphoglycerate}]} K$$

Attempts were made to upset equilibrium. The [lactate]/[pyruvate] ratio was rapidly changed by injection of ethanol or crotyl alcohol, and the value of [ATP]/[ADP][HPO<sub>4</sub><sup>2-</sup>] was rapidly changed by injection of ethionine or carbonyl cyanide *p*-trifluoromethoxy-phenylhydrazone. 2. The concentrations of the metabolites occurring in the above equation were measured in freeze-clamped liver. 3. Although the injected agents caused large changes in the concentrations of the individual components, near-equilibrium in the system was maintained, as indicated by the fact that the value of [ATP]/[ADP][HPO<sub>4</sub><sup>2-</sup>], referred to as the phosphorylation state of the adenine nucleotides, measured directly agreed with the value calculated for equilibrium conditions from the above equation. 4. The results are discussed and taken to confirm that the order of magnitude of the value of the redox state of the cytoplasmic NAD couple in rat liver is controlled by the phosphorylation state of the adenine nucleotide system.

The redox state of the NAD couple is linked to the phosphorylation state of the adenine nucleotide system (*i.e.* the ratio [ATP]/[ADP][HPO<sub>4</sub><sup>2-</sup>]) by the 3-phosphoglycerate kinase and glyceraldehyde 3-phosphate dehydrogenase reactions (Veech *et al.*, 1970; Krebs & Veech, 1970). At equilibrium the following relation holds :

$$\frac{[\text{NAD}^+]}{[\text{NADH}]} = \frac{[\text{ATP}]}{[\text{ADP}][\text{HPO}_4^{2-}]} \cdot \frac{[\text{3-phosphoglycerate}]}{[\text{glyceraldehyde 3-phosphate}]} \frac{1}{K} \quad (1)$$

where  $A$  is the product of the reaction between the p-mercaptoaldehyde 3-P and the oxidizing agent, and  $K$  is the equilibrium constant of the system  $2H^+$ . By combining equation (1) with equation (2), we can derive the following equation, which can be tested experimentally:

$$\frac{ADP}{ADP + H_2O} = \frac{K}{K + 1} \frac{A}{P} \quad (3)$$

where  $A$  represents the product of the reaction between the p-mercaptoaldehyde 3-P and the oxidizing agent, and  $K$  is the equilibrium constant of the system  $2H^+$ . By combining equation (1) with equation (2), we can derive the following equation, which can be tested experimentally:

The present work (Guha *et al.*, 1972) was carried out to determine the normal and abnormal levels of the enzyme 3-P dehydrogenase in rat liver and kidney. The results are presented in Table I. The enzyme activity was found to be higher in the liver than in the kidney, and the activity was found to be higher in the normal than in the abnormal animals. The results are discussed in the following sections.

## EXPERIMENTAL

### Rats

Female rats of the Wistar strain weighing 150-200 g were used except in the ethanol experiments, where adult male Sprague-Dawley rats were used. All were stored under standard conditions.

### Reagents

Standard analytical grade reagents were obtained from British Drug Houses Ltd. P-mercaptoaldehyde 3-P and NAD were obtained from Boehringer Corp. Ltd. and NADH was obtained from D. P. G. Hewer Ltd. Lu Pont de Nemours and Co. Ltd. Central Research Department, Experimental Station, Wilmington, Del. U.S.A.

### Infection of agents



Although ATP] was an increase (4.3%) and  $\Delta$ ATP] decreased (1.6%) in ethanol-treated animals.

In spite of minor changes in the above parameters, the overall degree of agreement between the control and ethanol-treated groups was very good. There was no significant difference in the values of  $\Delta$ ATP] and  $\Delta$ ADP] between the two groups.

### Effect of crotyl alcohol

Crotyl alcohol was given to the rats in a dose of 0.5 ml/kg body weight. The rate of conversion of pyruvate to lactate was not significantly different from the control rate of conversion of pyruvate to lactate (Table 2).

The changes in the concentrations of pyruvate, lactate, and crotyl alcohol (Table 2) are given in Table 2. The values of the  $\Delta$ ATP] and  $\Delta$ ADP] are given in Table 2.

TABLE 1. The effect of ethanol on the rate of conversion of pyruvate to lactate in the liver of rats. The values are the mean  $\pm$  S.E. of the mean.

Group	Pyruvate ( $\mu$ moles/g liver)	Lactate ( $\mu$ moles/g liver)	$\Delta$ ATP] ( $\mu$ moles/g liver)	$\Delta$ ADP] ( $\mu$ moles/g liver)
Control	0.12 $\pm$ 0.01	0.12 $\pm$ 0.01	0.12 $\pm$ 0.01	0.12 $\pm$ 0.01
Ethanol	0.12 $\pm$ 0.01	0.12 $\pm$ 0.01	0.12 $\pm$ 0.01	0.12 $\pm$ 0.01

TABLE 2. The effect of crotyl alcohol on the rate of conversion of pyruvate to lactate in the liver of rats. The values are the mean  $\pm$  S.E. of the mean.

Group	Pyruvate ( $\mu$ moles/g liver)	Lactate ( $\mu$ moles/g liver)	$\Delta$ ATP] ( $\mu$ moles/g liver)	$\Delta$ ADP] ( $\mu$ moles/g liver)
Control	0.12 $\pm$ 0.01	0.12 $\pm$ 0.01	0.12 $\pm$ 0.01	0.12 $\pm$ 0.01
Crotyl alcohol	0.12 $\pm$ 0.01	0.12 $\pm$ 0.01	0.12 $\pm$ 0.01	0.12 $\pm$ 0.01

Metabolite or metabolite ratio	Control (4)	Ethanol-treated (5)	P
Lactate	0.28 $\pm$ 0.02	0.65 $\pm$ 0.45	<0.05
Lactate	0.021 $\pm$ 0.006	0.028 $\pm$ 0.008	>0.1
Lactate	12.6 $\pm$ 1.72	97.9 $\pm$ 8.14	<0.05
Lactate	0.06 $\pm$ 0.01	0.013 $\pm$ 0.004	<0.1
3-Hydroxybutyrate	0.17 $\pm$ 0.03	0.08 $\pm$ 0.02	<0.05
3-Hydroxybutyrate	0.11 $\pm$ 0.03	0.09 $\pm$ 0.04	<0.1
3-Hydroxybutyrate	1.66 $\pm$ 0.21	1.4 $\pm$ 0.41	<0.1
Acetoacetate	0.13 $\pm$ 0.03	0.47 $\pm$ 0.11	<0.025
3-Hydroxybutyrate	0.02 $\pm$ 0.02	0.0 $\pm$ 0.00	<0.05
Acetoacetate	0.02 $\pm$ 0.02	0.02 $\pm$ 0.01	<0.001
ATP	2.25 $\pm$ 0.05	0.17 $\pm$ 0.01	<0.001
ADP	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	<0.001
P <sub>i</sub>	4.83 $\pm$ 0.05	0.0 $\pm$ 0.00	<0.05
(NAD <sup>+</sup> - NADH) / (NAD <sup>+</sup> + NADH)	0.15	0.03	
(ATP - ADP) / (ATP + ADP)	0.0	0.0	
(ATP - ADP) / (ATP + ADP)	0.0	0.0	

The two alkyl phosphates, 3-phosphatyl-3-phosphoglycerate (1a) and 1-phosphatyl-3-phosphoglycerate (1b) were prepared from 4,9-bis(2'-phosphoryl)-9H-thioxanthene (2) whereas 1-phosphatyl-3-phosphoglycerate (1c) was prepared from 1,3-bis(2'-phosphoryl)-3-phosphoglycerate (3). The yields of 1a, 1b, and 1c were 40, 40, and 40%, respectively. The agreement between the observed and calculated proton ratios in these compounds was good.

[illegible]

The following table shows the distribution of the population of the United States by race and sex in 1900. The population of the United States in 1900 was 76,212,367. The population of the United States in 1910 was 92,228,496. The population of the United States in 1920 was 106,011,273. The population of the United States in 1930 was 122,765,958. The population of the United States in 1940 was 136,623,226. The population of the United States in 1950 was 150,697,361. The population of the United States in 1960 was 179,323,216. The population of the United States in 1970 was 203,211,926. The population of the United States in 1980 was 226,545,804. The population of the United States in 1990 was 253,699,572. The population of the United States in 2000 was 281,421,906. The population of the United States in 2010 was 309,292,388. The population of the United States in 2020 was 331,449,281.

Metabolite	Control	Formycin-treated
ratio	(22)	(40)
1. Glucose	0.00 ± 0.02	0.77 ± 0.09
2. Fructose	0.00 ± 0.00	0.17 ± 0.01
3. Inositol	14.2	45.3
4. Glycerol	0.002 ± 0.001	0.002 ± 0.001
5. Phosphoglycerate	0.16 ± 0.02	0.022 ± 0.003
6. Pyruvate	0.18	0.11
7. Phosphoenolpyruvate	1.81 ± 0.11	1.85 ± 0.17
8. Lactate	0.00 ± 0.00	0.24 ± 0.02
9. Hydroxybutyrate	0.8	7.2
10. Acetate	2.12 ± 0.08	2.28 ± 0.05
ATP	1.48 ± 0.1	1.11 ± 0.04
ADP	1.00 ± 0.1	1.88 ± 0.10
P	0.31	1.18
NAD <sup>+</sup> /NADH (measured)	7.24	2.39
NAD <sup>+</sup> /NADH (calculated)	7.00	1.89
ATP/[ADP][P] (measured)	628	1181
ATP/[ADP][P] (calculated)		

## Effect of carbon tetrachloride poisoning on the cytochrome P-450 system in the liver of the rat

This compound is an uncoupler of oxidative phosphorylation, is expected to decrease the value of the phosphorylation state of the adenine nucleotide system. The effects of the inhibitor on concentrations of nucleotides showed considerable quantitative variations but they were always of the same kind. These variations were due to the fact that the effects of the inhibitor were very rapid. Thus [ATP] fell to  $10^{-4}$  M in 2 min. With the dose













# A PRIORI APPROACH TO THE SOLUTION OF BIOLOGICAL PROBLEMS

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During the past several years our laboratory has concentrated on two main problems—the molecular principles of membrane construction and the mechanism of energy transduction in biological systems. Our efforts on each of these two problems have followed a similar course. First we embarked on a search for a principle and then proceeded on a program of verification, versatile to rationalize the existing body of experimental information. The fundamental principle for membrane construction (1-3) and the electromechanochemical principle for energy transduction (4-5). As we have reflected on the approach which was taken in arriving at the respective principles and in testing the accommodation of the experimental data to these principles we recognized that we had introduced a new dimension in the nature of problem solving. The present article is addressed to this new dimension which we have created to refer to as the *a priori* approach to the solution of biological problems.

## 1. THEORY OF THE *a priori* METHOD

There are two approaches to the solution of scientific problems—the classical or inductive method and the *a priori* or deductive method. Scientific problems may be divided into two classes—those which can be solved by the inductive method and those which cannot at any given period. The latter problems can only be solved by the *a priori* method. The inductive method involves extracting the correct conclusion from a series of experimental observations based on established physical principles and rigorous logical reasoning. Of primary importance in the application of the inductive approach are the rigorateness of the physical principles invoked and the soundness of the logical processes. Given these two conditions, the correct conclusion follows automatically from experimental facts just as input data can be transformed into output information in a predictable manner by an electronic computer. We will refer to scientific

problems which are not being solved, and to those which can be solved. Some of the problems are the lack of a clear capability to distinguish between the important problems and the less important ones, and the lack of a clear

[illegible][illegible]





[illegible]

## II. GENERALISTS AND THE *q* METHOD METHOD

[illegible]



The manner in which the model would have been suggested is of course the subject of the search for a better model. Such a search is a matter of fact, and has been because. Problem solving requires intelligent and rigorous experimentation. The tactical component in research is creative. It is for this very reason that the *a priori* method of problem solving plays a role in the scientific effort. For example, in the case of a problem, it is not up to the fact, but of content, and it is not up to the fact, but of content and theory. Experimentation is a matter of fact, and it is a matter of fact which has become superannuated by the facts.

Perhaps a more fundamental way to distinguish these capabilities is to consider the directionality of the method. This directionality is positive when the method is high in the latter. One can expect a method to be useful without thinking about solving problems but it is unlikely to be a procedure for a problem without thinking about one or more problems. In that sense, the program method has a directional bias, lacking in the classical inductive method.

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## ACADEMIC ASPECTS OF APPLIED RESEARCH ON FOOD PROTEINS

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In every country, regardless of its state of development, biological research should, among other things, be useful. Each scientist engaged in it should be ready to explain comprehensively and convincingly how what he is doing may be expected to improve the nutrition, health or comfort of the community. Quakers who found the "Pragmatic School" in the 18th century maintained that the whole of society was parasitic on farmers—that may be an extreme position. But it is undeniable that food is a more fundamental requirement than clothing or housing and peoples' food needs are much more anxious than their real or imagined medical needs. It would be reasonable, therefore, to give precedence to the study of food production in the hope that by the end of this century the supply will be adequate throughout the world.

Although we must believe its primary purpose, research will not be successful if it is planned too narrowly. Both for the intellectual satisfaction of the scientist and for the logical development of the subject, some exploration in depth is reasonable. Furthermore, the work need not necessarily be planned so as to yield practical results, though it may eventually work out, even on a highly applicable basis, in a way that is not foreseen. The appropriate division of effort between basic and applied research will have to be decided by the work with long-range application and work undertaken so immediately the subject will depend on the resources that each community is prepared to devote to research.

In technically developed countries the amount of money per head spent on applied research is greater than in the developing countries. But because of greater affluence so much more basic research is also done that it tends to dominate the research scene. Much of this impractical research shows little originality and may not even present much evidence for the scientific competence of those in need of it. One gets the impression that a certain species of enzyme is being studied for no better reason than that no one has hitherto taken the trouble to study it. This is what, in one might say, clutter—the literature. It is unfortunately often taken as a model of what scientific research should be. Research does not become original merely because it is concerned with something hitherto uninvestigated if the objective methods and concepts are



all conventional. There is admittedly a random element. Something novel may emerge and be noticed. This is true as it is in basic — thoroughly practical research. Especially in a new environment. A country without extensive research establishment should be wary of following the bad example set elsewhere. Only the wealthy can afford to run a scientific band wagon. A real difficulty arises over who is to judge a discovery satisfactory. Science is self criticism. How practical this criticism is debatable. No one would wish to discourage an earnest research worker who has a genuinely original concept or method. However, he who simply heuristics and is called to call for more evidence to get it accepted is then is usually called for in affluent countries. Judging from the number of genuine discoveries in affluent countries, it would not be reasonable to expect more than 2 — 3 for each million of the population.

Experience suggests that there is a correlation between the equipment and attitude of scientists and their ability to get the very best results with simple equipment. In all countries most of the problems of a scientific absence of expensive equipment come from lack of equipment. This may be safeguarded against by blaming the lack of equipment on lack of productivity. Biological administrators have sometimes suggested that productivity could be stimulated by widening the conditions for research. That is obviously absurd. The fact is that a country's productivity depends on the people who are doing it rather than on the equipment with which it is done. Without certain types of equipment certain types of research cannot be undertaken. But there are other types of research that are worth bearing in mind that the research on which the present agricultural prosperity of Western Europe and USA depends was done with primitive and poor equipment now seen primitive. It is also worth bearing in mind the many of the failed attempts to set up self-sustained European settlements in Asia and USA countries now embarrassed by food supplies failed because of starvation. There is, therefore, no reason to think that countries that have got into the habit of importing much of their food will not become self-sufficient when adequate applied research is done. This research is often approached unreasonably timidly. The productive attitude is that this problem will be solved at some time or another. It might as well be solved by the new

The early emigrants from Europe, coming with the pioneers to the countries to which they were trying to establish settlements, had an immediate advantage over those who are now trying to improve agricultural standards in somewhat similar countries. They were compelled to be self-sufficient because there was no one else to whom they could turn for help. Had there







for even his initial experiments. The point here is very plain. A protein rich yam could be very useful, but it would not completely solve the problem of erosion because it would not give complete ground cover throughout the year.

In many parts of the world, yams are used to prevent erosion—but they are far from ideal. Yams even wanted. Research directed at finding a protein rich food crop for erosion control was started over is urgently needed. I do not know what the best part of what can be done without more protein rich crops is. I do not know. The yield from such a crop would probably be small. A crop in which the protein can be extracted and used for other purposes would be of more use. When Guha was working in Canada, he was of some because he had no unwillingness to be motivated by a more exacting target. I can also remember with a certain immediate problem, but the value of scientific research is more general than the work he was doing on which he was engaged and the work he was doing. I was working. The need for a new crop protein was not at all clear. When the war caused a slight food shortage in Britain, it was caused by food shortages in India, we both began to plant protein rich food crops. Other responsibilities descended Guha from the work, but I have continued it.

At R. I. and we began to study of extracted dry protein from a beetroot leaves. Around 1970, he studied it. My work should be possible to produce a protein rich food crop. I had a period of good weather. The process of extraction and purification is simple enough to be used in a small field and the product is better than most available protein rich food crops (see papers on the subject in Proc. 1969-1971). Careful attention to work was needed to design systems of husbandry that will give optimum yields. This has started in Aurangabad, Coimbatore and elsewhere. It is reasonable to expect many intriguing new phenomena in plant physiology to emerge when serious effort is put into attempts to maximise vegetative growth.

The possibility of using leaf protein as a human food broadens the basis of crop production. Work on maximizing protein value and on rate reduction was high to not practically useful unless the protein is in a form suitable to people or stock, or in a form that is effective in converting the protein into seeds or tubers. Even when it is in a form suitable for use where the protein can be taken place, the protein is not a food. The protein must be readily extractable by the method now used or envisaged. Thus, whether or not an understanding is the use of leaf protein, but when the leaves

are large enough to give a useful harvest, the protein extract is poor. Poor extraction can arise in different ways from many different causes. Sometimes the trouble is physical and could be overcome by more vigorous pulping or by digestion with cellulase or other enzymes (Pace, 1953). Acid causes such as mold might be a factor in preventing the penetration of alkali. Extraction from the more fibrous stem plants is more difficult and proceeds unhindered by pulping and the use of some agents. The difficulty may be circumvented by using a detergent which is not a surfactant. While the latter is not of interest in itself, it is of practical importance. Cellulase is also responsible for the reduction of protein in the plant, and together with the plants are now often damaged by the action of the enzyme. Cellulase is also responsible for the reduction of protein in the plant, and together with the plants are now often damaged by the action of the enzyme. Cellulase is also responsible for the reduction of protein in the plant, and together with the plants are now often damaged by the action of the enzyme.

The essence of the present work is to show that the work which is now done on plant breeding for feed purposes and on the use of the various useful and harmful substances in the feed is not merely a collection of the protein shortage and a collection of the various uses of plants will contribute to reducing it. The present work is a guide to the work which is the usual departmental discussion of a plant and a reasonably competent student should be able to get the results which are of interest. Some other particularly useful lines of work would be to have a more systematic

Several publications suggest that a particular variety or mixture of plant species or varieties can be used as a plant with any use. The plant should be chosen up because it is of great value and is not a waste of space. Detergent exudates and effluents are well known and there is a need for more growth in natural conditions. Herbert's work on the subject has been inadequately replicated and controlled. Any research now undertaken should be impeccable.

Tropical waterways are constantly threatened by water weeds. It is widely assumed that these weeds are serious and that cutting or the use of herbicides are the only satisfactory means of keeping canals and irrigation ditches clear. These assumptions may not be valid. The water hyacinth is being collected as cattle and pig food and I have seen these animals browsing readily on the weed in a fenced pond. There is no doubt that it is no official. It would be easy to get the weed as a feed material, treating part of a conventional diet to pigs or running with hares and studying the effect on performance. It may be that the hyacinth would need pre-treatment, e.g. chopping, crushing or heating. This work could be done on a small scale in form





countries that need extra food—they were busy with more remote and impractical aspects of plant physiology. It could indeed be regarded as scientific colonialism—perpetrated in a good cause. One reason for this which is regularly commented on and deplored is the tendency of scientists returning to a developing country after a period of training elsewhere to try to continue with the line of work they had been following in the affluent country. A more serious cause is lack of sufficient appreciation of present and other biological potentialities. Students follow this bad example. Fewer than 5% study agriculture in the universities in developing countries or in the universities abroad to which they go for further training. For as long as villages are neglected and governments think of development in terms of urban industrialisation this attitude of the students is reasonable. They regard education as a way to escape from village life—not to improve it.

The tendency to hold agriculture—and even science in general—in low esteem is not confined to developing countries. According to the US President's Science Advisory Committee report on 'The World Food Problem' (1967) scientists engaged in agriculture in the USA are paid less than any other group of scientists and in the British civil service an administrator is seven times as likely to get £5000 a year as is a scientist (Probit 1967). But things may be changing. The Minister of Agriculture of one developing country was recently quoted as saying: "Now I can get the Finance Minister to return my phone calls."

A sound principle governing research in developing countries is: "if the work could just as well be done in an affluent country—it should be done there." The laboratory space and human skill of developing countries will produce the most useful and original results when applied to problems peculiar to these regions. But the final presentation must be complete and unassailable. There have been enough hurried and unconvincing 'preliminary communications'. What is needed is thorough investigation so that there will be no further argument on the matter. Young scientists are sometimes unwilling to undertake such thorough work because after several years they will have only one paper to show for it whereas the same amount of effort put into routine research might have produced—or at any rate permitted sub-division into—a dozen papers. Any tendency in a University to assess merit by the number of publications must be roundly condemned. Any fool can split a piece of work into little bits and get them published separately; only an able scientist can produce a complete and unassailable statement. That proposition has universal application—it has nothing to do with the state of development of a country.

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circulation system added to her diagnostic assistance. Biochemistry, faced with new compounds having different physical and chemical characteristics, tends to be concerned with some of the following matters:

(i) *Biosynthesis*—When the compound is derived, for example, from a plant, its biosynthesis is a matter of great interest. It poses many problems. Biochemistry may devise experimental approaches using isotopically labelled starting precursors so that intermediates may be isolated and characterised using inter alia several spectroscopic methods.

(ii) *Function*—Many new compounds are isolated because a biological effect has been discovered first. Monitoring by bio-assay, applied classically to compounds of known activity, is now evident in research on pheromones. In other areas compounds have been isolated without the help of any biological response and functions have to be looked for. Antibiotics have been screened and useful properties revealed but the study of function within the whole organism is now at various stages. It should be noted that some compounds are known to act as specific inhibitors, e.g. at defined stages in the respiratory chain, and such compounds may be more valuable as research tools than as therapeutic agents.

(iii) *Metabolism*—Ingested compounds undergo change within living organisms, e.g. amines can be 'detoxicated' to ether substances in the liver (although the metabolism can sometimes result in increased toxicity).

(iv) *Pharmacological properties*—New substances may possess 'beneficial' effects but before they can be brought into wide use they need to be tested for toxicity, carcinogenicity, teratogenicity and mutagenic potency.

(v) *Detailed mode of action*—Biochemistry is now rich in arrays of metabolic patterns and once a biological property has been defined as due to a new substance the evidence needs to be fitted into a varied metabolic scheme or schemes.

Analytical chemistry has reached a new level of sophistication and very small amounts of 'active' substances can often be determined. The implications for biochemistry are very broad. Two may be mentioned here, first that analytical biochemistry must be kept up to a similar standard and second that the selection of problems for biochemical study becomes increasingly critical when programmes could be envisaged almost indefinitely.

The above lines of thought can be illustrated by the role of spectroscopy in different phases of research particularly on unsaturated compounds.



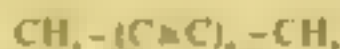
### 1. Poly-enes, poly-ynes and poly-enynes

(a) The ultraviolet absorption spectra of linear conjugated poly-enes  $R-(CH=CH)_n-R'$  show weak near 220nm ( $\lambda_{max}$  200-220) when  $n=2$ , and as  $n$  increases the absorption is shifted to longer wave-lengths, e.g. to 330, 340, 360 and 375nm ( $\lambda_{max}$  300-350). A carboxyl group has an additional bathochromic effect. Thus,  $H_2C=CH-(CH=CH)_nCOOH$  shows  $\lambda_{max}$  260nm. A weak absorption band of  $CH_2=CH_2$  is clear.



There is marked difference in spectra of conjugated poly-enes and it falls into a clear pattern.

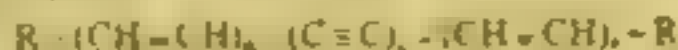
(b) The spectra of poly-ynes present a similarly coherent picture. The family of compounds



exhibits a weak absorption band and a second set of very strong bands. The strong bands occur at shorter wave-lengths, e.g. the decatriene shows a very strong peak at 2600nm ( $\lambda_{max}$  352000) with subsidiary peaks at shorter wave-lengths with separations of 2000-2300nm<sup>-1</sup>. The weak peaks, showing larger separations, occur between 300 and 394nm with  $\lambda$  values 100-500.

The hexatriene and heptatriene spectra are basically similar, the location of each of the two groups of bands showing a bathochromic shift for an increase in  $n$ . The intense bands show big increases in  $\epsilon$ , as  $n$  increases but the weak long wave bands do not. The positions, intensities and separations ( $\lambda_{max}$ ) are all diagnostic of the chain length of poly-ene chromophores.

(c) Compounds that contain conjugated olefinic and acetylenic groups exhibit similar resolved absorption spectra. Sufficient compounds of known structure have been examined to allow  $\lambda_{max}$  and  $\epsilon$  to be identified from the spectra of new poly-enynes.

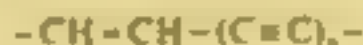


(Boulton et al. 1952, 1953a,b; Hauser et al. 1955; Naxler and Whiting 1954; Jones et al. 1960).

### 2. Unsaturated fatty acids

Unsaturated acids with two, three or four conjugated double bonds are 9, 11-moleic acid ( $\lambda_{max}$  32,500),  $\alpha$ -erythroic acid ( $\lambda_{max}$  nm 262, 271.5, 283, 36,200, 45,100, 37,300 in cyclohexane) and  $\alpha$ -parinaric acid ( $\lambda_{max}$  nm 293, 305, 319,  $\epsilon$  50,000, 77,300, 69,100 in cyclohexane). The displacements in the wave-length and intensity scales are consistent and diagnostic. Signi-

larly the spectrum of stillicic acid fits its structure as a diene conjugated with the carboxyl group. X-methylene acid (octadec-11-en-9-yne-8-oic acid) shows the expected 229nm peak. Erythrogenic acid (—dec-17-ene-9-11-diene) shows in ethanol three peaks at 227, 228 and 252nm ( $\epsilon$  37, 344 and 12) respectively. Cupillene (CH<sub>3</sub>—C=C—C=C—CH<sub>3</sub>—CH<sub>3</sub>) shows peaks at 249 and 253nm ( $\epsilon$  537, 427) the absorption due to the phenyl group being masked and the CH<sub>3</sub> interrupting conjugation. The diene group in erythrogenic acid is evidently insulated from the carboxyl group by CH<sub>2</sub> groups. Balaic acid (octadec-7-ene-17-diene) acid which has peaks at 227, 240, 253, 267, 282.5nm ( $\epsilon$  3060, 6300, 11700, 7400, 3600). Isopropyl acid (octadec-11-ene-4-ene-12-diene) acid) has very similar maxima, 227, 240, 253, 267, 282.5nm and must contain the chromophore.



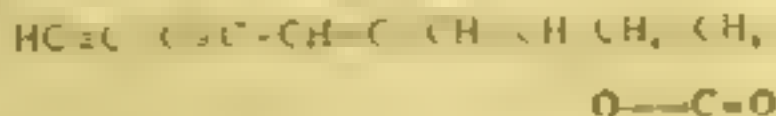
but the two acids may not have the same structure

Acthuvon  $\text{CH}_3\text{CH}=\text{CH}-\text{C}(\text{CH}_3)_2-\text{CH}=\text{CH}-\text{CH}_3$ ,  $\text{CH}_3$  from  $\text{Ar}=\text{H}$  and  $\text{Ar}=\text{CH}_3$   
 plus 1, shows the following spectrum

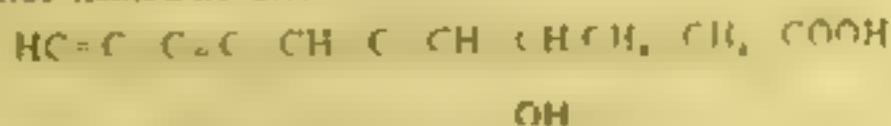
$\lambda_{\text{max}}$ , nm.	249.	266	277.	295.	313.	335
$\epsilon \times 10^{-3}$	1.16	2.69	1.66	2.88	3.95	2.84

The *uvohio*, at least, shows a continuous spectrum (Pitt and Morton 1957; Yamiguchi 1957).

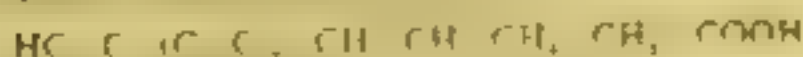
Bu'Lock *et al.* (1955) measured absorption spectra for the anhydrous nemolin and its dimer,  $\epsilon_2$ . Nemolin is an unsaturated acetone



which yields nemounic acid



and on treatment with alkali removed A



The absorption peaks are as follows :-

Nematode	$\lambda_{\text{max}}$ nm	20°C				24°C			
		$\epsilon \times 10^{-3}$	$\epsilon_{\text{max}}$	$\lambda_{\text{max}}$	$\epsilon_{\text{max}}$	$\epsilon \times 10^{-3}$	$\epsilon_{\text{max}}$	$\lambda_{\text{max}}$	$\epsilon_{\text{max}}$
Nematode A	204	21	240	241	268	272	288	307	328
Nematode B	204	23	59	85	28	6	12	15.5	11



Mycomycin (Calmer and Solomons 1952, 1953) has the formula  $C_{19}H_{26}O_4$  and displays its evident conjugable unsaturation in a simple but strong spectrum:

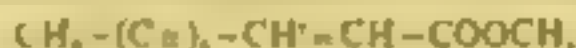
$\lambda_{max}$ , nm	256	267	361
$\epsilon_{max} \times 10^{-4}$	3.3	6.1	8.7

Alkali isomerises mycomycin to isomycomycin which exhibits a much more complex absorption spectrum:

$\lambda_{max}$ , nm	246	267.5	267	267.5	304.5	324	347
$\epsilon_{max} \times 10^{-4}$	2.4	5.9	11	14	2.7	4.1	3.4

The infrared absorption of mycomycin has peaks at  $3100\text{cm}^{-1}$  ( $\text{CH}$ ),  $2200\text{cm}^{-1}$  ( $\text{C}\equiv\text{C}$  disubstituted),  $1930\text{cm}^{-1}$  ( $\text{C}=\text{C}-\text{C}$ ) and  $1730\text{cm}^{-1}$  ( $\text{COOH}$ ). Isomycomycin has lost the  $930\text{cm}^{-1}$  peak but the  $730\text{cm}^{-1}$  peak persists. In mycomycin the  $1130\text{cm}^{-1}$  (or the  $267\text{nm}$  peak in some) comycin points to a cyclopropane structure.

Transdehydroretene ester (Bohlmann and Manbhardt 1955)



shows a similar spectrum:

$\lambda_{max}$ , nm	244.7	255	268	267.5	304.4	329.374	341.5
$\epsilon_{max} \times 10^{-4}$	7.18	10.63	8.6	1.5	3.3	4.3	6

The allene group (Calmer and Solomons 1953) does not function like a conjugated diene:

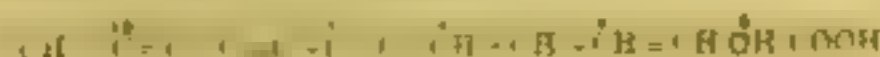
	$\lambda_{max}$ , nm		$\lambda_{max}$ , nm
$\text{R}^1\text{H}-\text{C}=\text{CH}$	170	$\text{R}^1\text{CH}=\text{CR}^2\text{H}$	186
$\text{RC}=\text{C}=\text{CH}_2$	210-215	$\text{RC}^1=\text{C}^2=\text{CH}$	212-214
$\text{COOH}$		$\text{COOR}$	
$\text{Ph}_3\text{C}-\text{C}=\text{C}=\text{CPh}_3$	267	$\text{Ph}_3\text{C}-\text{C}=\text{C}=\text{CPh}_3$	250
$\text{C}_6\text{H}_5-\text{CH}=\text{C}=\text{CHPh}$	208	$\text{C}_6\text{H}_5-\text{H}-\text{CH}=\text{C}=\text{CHPh}$	205

Mycomycin has the structure:



and it exhibits two separate chromophores, an ene diene and a triene, acting additively.

Isomycomycin with the structure



is a conjugated triene-diene

$\lambda_{max}=257\text{nm}$



and D-Lee, 1970; H. Lee *et al.*, 1971). Studies of ultraviolet spectra in the ultraviolet and infrared regions can easily multiply by probing over the entire D range of F and D (Fig. 2). The presence of carotenoids, in addition to the carotenoids found in plasma, can be detected by ultraviolet irradiation. The presence and absence of carotenoids is of considerable diagnostic value.

Ultraviolet D range carotenoids are found in the plasma and the carotenoids are found in the plasma and the carotenoids are found in the plasma. Both the low and high carotenoids are found in the plasma. Vetter *et al.* (1971) contributed a large volume of data on the carotenoids. An increase in the amount of carotenoids in the plasma and the carotenoids have been reduced to zero. This suggests that the carotenoids A and A<sub>2</sub> and their derivatives. Infrared spectra of carotenoids have been used to identify acetylenic and a cyclic group. The infrared peak of carotenoids indicates an acetylenic group and the 2.2  $\mu$ m peak of carotenoids characterizes an acetylenic carotenoid.

NMR spectroscopy has been applied with vigour in the carotenoid field and the system used information has been used to make the investigation of structure in a new carotenoid almost a routine. A point of special interest is that recent work in NMR spectroscopy has made it possible to be made of the percentages of the 6 MHz, 10 MHz and the 22 MHz instruments—a matter of importance when money for equipment is in short supply!

The significance of its role in some of the carotenoid field was first elaborated by Zechmeister and extended later (see Barber *et al.*, 1962; Weedon, 1971). The 220 MHz instrument permits for many polymers a resolution of the olefinic part of the spectrum so that the position of almost every proton can be ascertained. This is significant point for vitamin A isomers and particularly so for the *cis-trans* forms of retinaldehyde. The 11  $\mu$ m forms of the aldehydes of vitamins A<sub>2</sub> and A<sub>3</sub> give rise to special problems.

Table

Chemical shift (in ppm) of carotenoids



All-trans-retinal

11-cis-retinal

Olefin position	All-trans		11-cis		9,13-diene	
	m <sup>2</sup> mole <sup>-1</sup>		m <sup>2</sup> mole <sup>-1</sup>		m <sup>2</sup> mole <sup>-1</sup>	
7	6.14	6.36	6.32		6.36	
8	6.09	6.17	6.14		6.68	+0.54
10	6.06	6.19	6.54	+0.35	6.16	
11	6.60	7.15	6.69	-0.46	7.16	
12	6.27	6.37	6.92	-0.45	7.25	+0.55
14	5.67	5.97	6.07	+0.10	5.87	-0.10
CH <sub>2</sub> 16	4.29					
CH <sub>2</sub> 9	1.97	2.03				
CH <sub>2</sub> 13	1.85	2.32				
Ultra-violet absorption $\times 10^4$	1.00	1.00	1.00		1.00	0.17
$\lambda_{max}$ 325nm.	361	376.6	373			

In this paper the stress has been on *carotenoid* polyenes and polyynes. The conjugated systems are however important in many ways. Thus the higher polyenes like deinoxanthin and fucoxanthin, zeaxanthin and the newer carotenoids etc. are essential to animal health but so far not fully studied by other spectroscopic methods. Carotenoid nutrition revealed by red and blue spectra. These are the precursors by synthesis of complex carbohydrate derivatives. The polymeric side chains of plastoquinones, ubiquinones and menaquinones are present in molecules needed for photosynthesis, electron transport and vitamin K activity (Maret, 1971).

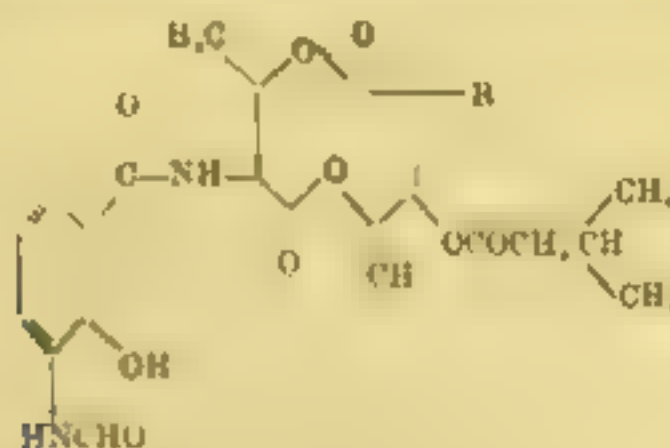
Nowadays examples occur where mass spectra fragmentation patterns have greatly helped in the elucidation of structure of such polyunsaturated compounds. Problems of biosynthesis in the carotenoid field have been the subject of highly successful efforts. The whole topic is reviewed in detail by Goodwin in Isler's very recent volume. There is nothing to be added here except that some recent developments illustrate how new and intriguing issues are arising as research proceeds.

Batra (1967a, b) observed that a non photosynthetic bacterium *Aerobacterium anatum* produced carotenoids only when illuminated. This photoinduction is made up of a temperature independent oxygen requiring photochemical phase followed by dark reactions needing oxygen but not light. It was also found that antimycin A could induce carotenoid synthesis in the organism in the absence of light. The induction effects of the antibiotic and of light were additive so that different sites of action could reasonably be postulated.

Antimycin A is well known as an inhibitor of the electron transport system of aerobic organisms including yeasts, fungi and animals (Räbergh 1967), the

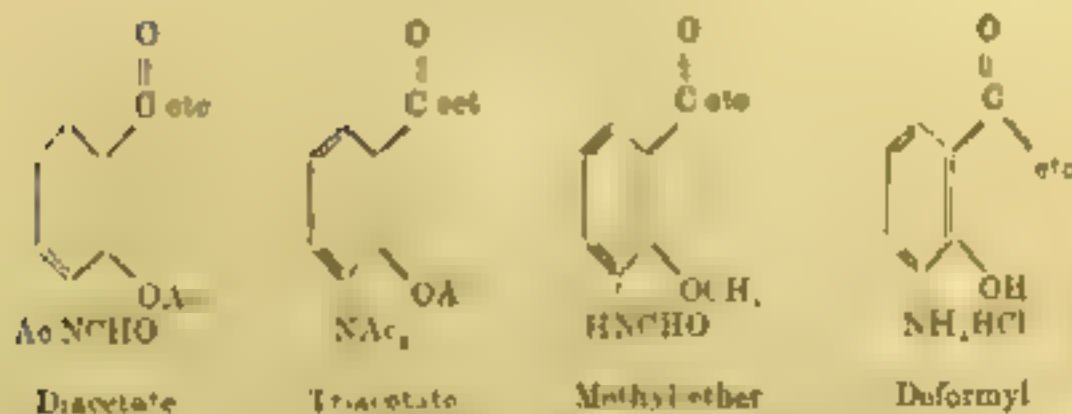
effect being located at the cytochrome level (Chance 1968). Antimycin A exists as a complex with four components all of which inhibit electron flow. A similar effect is also obtained with 2N-heptyl-4-hydroxyquinoline-N-oxide but that compound does not induce carotenoid biosynthesis in *M. marinum*.

Batra *et al* (1971) made a study of the relationship between structure and activity in the antimycin-induced carotenoid synthesis. Antimycin A consists of four compounds each of which was as active as the antimycin A complex as a whole (optimally at  $40 \mu\text{M}$ ) and in each case light enhanced the effect additively. [The components differ in respect of the alkyl substituent in the dilactone ring]

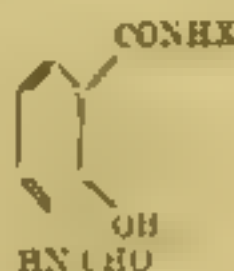


R = Heptyl Antimycin A<sub>1</sub>

R = Butyl Antimycin A<sub>2</sub>



The chromophoric portion of the antimycin molecule is the aromatic 3-formamidosalicylamide moiety:



but a dozen synthetic compounds differing in respect of the group X (hexyl, octyl, decyl, phenyl, benzyl, etc.) failed to induce carotenoid biosynthesis from which was concluded that the group X was not important. Further, more intensive dissection and analysis of the *car* gene failed to show, although they may have been unsuccessful, that the *ph* gene and *P*-protein did not reduce the activity of the *N*-terminal group as a co-receptor for inducing carotenoid biosynthesis. The *car* gene is also responsible for the enhancer effect in electron transport.

The methyl ether was synthesized and was checked by nuclear magnetic resonance (the downfield resonance due to the phenyl proton was replaced by an intense signal at 6.1 due to the methoxy protons) and it failed to induce carotenoid synthesis in the dark. Surprisingly it stimulated light-induced carotenoid synthesis at least three-fold.

The studies of carotenoid synthesis induced by retinoids. A 4-day lag period of about 4 hours is observed in *Chlorella* in the presence of some known inhibitors of protein synthesis and carotenoid synthesis. Carotenoid production (Bauer and Dreyer, 1969; Murakami *et al.*, 1970) is induced by protein synthesis in *Tetrahymena* pretreated in the dark (Hatch, 1970). Hence it seems probable that both light and retinoids induce carotenoid synthesis by depressing the genetic sites controlling the synthesis of rRNA and the enzymes needed for carotenogenesis. The lag period observed with the methyl ether plus light could have been due to protein synthesis of other light-inducing enzymes. Addition of chloramphenicol gave evidence consistent with decrease protein synthesis following induction and blocking of carotenoid synthesis.

When these very interesting lines of research are pursued over many problems arise: (1) what is the absorbing entity in dark grown *Chlorella* which initiates the photo-induction of carotenoid production? (2) does the organism make any carotenoid precursors when grown in the dark? (3) are any colourless precursors of carotenoids synthesized during the lag period? (4) inasmuch as a number of enzymes are needed for biosynthesis of the carotenoids and their precursors do both light and retinoids depress several genetic sites? These comparisons are difficult to make due to the facts concerned but it will be noted that the implications are interestingly biochemical.

This review illustrates how spectroscopic techniques have contributed to a transformed approach to structure, biosynthesis and function. Biochemists not only need fine instruments and good technicians they also need to master the essential parts of a vast body of information that has been patiently gathered and reduced to order. The time is ripe for penetrating biochemical studies.



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# IMPROVEMENT OF NUTRITION DURING POSTWAR ECONOMIC DEVELOPMENT IN JAPAN

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The Second World War drove the Japanese people to the edge of starvation and occurrence of a large number of malnutrition patients. But after the War the food supply recovered to a normal level in a relatively short period of time and moreover some improvement in the eating habits has been made quite smoothly. At present we seldom see cases of deficiency of certain nutrients. We can see the change in food supply per person per day since 1930 according to the data provided by the Ministry of Agriculture and Forestry. Within 60% of the calories supplied by rice before the War. After the period of low caloric intake and fasting after the War, the calorie supply was recovered to the pre-war level in 1955. The rate of calories supplied by rice, however, did not reach the pre-war level and it has begun to decrease soon. At present rice gives 41% of the total intake of calories. On the other hand the variety of other cereals has increased a little compared with the pre-war level. The chief amount of these cereals consists of wheat in a form of bread, the use of which has been recommended through the school lunch program practiced in the whole country. The distribution ratio of calorie supply by cereals is decreasing year after year and is now about 1/3 of the total calories. During and directly after the War potatoes and sweet potatoes were consumed in large quantities, but this trend has been decreased in the recent ten years. Despite the decreased consumption of rice and potatoes, the total calorie intake is increasing slightly every year because of the increased consumption of starch, sugar, fats and oils and other foods. Pulses remain almost constant, but the supplies of vegetables and fruits have increased. Fish and shellfish remain constant, but the supplies of milk, meats and eggs have greatly increased in recent years.

As for the protein supplies, a remarkable increase occurred after the War. The per capita protein supply per day was 54.55g before the War, and 36g directly after the War, that is, in 1946. In 1950, 51g recovered the pre-war level and after that it increased year after year. It is now 59g and this increase is largely due to the increased supplies of meats, eggs, milk and milk products.



the mean weight of 1948 and that of 1968 are compared, the mean value except for the 13-year group around 25 in females, and the weight values do not go up in the older age both in 1948 and in 1968.

We do not think that the above mentioned increase of the physique is entirely due to the improvement of nutrition, but was produced by the positive correlations existed between the increase of energy intake and the intake of fat, animal protein, calcium, riboflavin and vitamins A, B<sub>6</sub> and C. Vegetable protein and carbohydrate showed no marked change.

We shall turn the topic to the present situation of food intake and economic circumstances. The latest circumstances of the Japanese people are reported by the Bureau of Statistics, Office of Prime Minister of Japan in the Annual Reports of the Food, Health and Hygiene Survey, 1968, in which we have the means of 1968 for each sex and age group. The mean energy intake per person per day was 2,600 kcal. (10,880 kJ) for males and 2,200 kcal. (9,180 kJ) for females in 1948 to 1949 and 2,700 kcal. (11,290 kJ) for males and 2,300 kcal. (9,580 kJ) for females in 1968. We can see that the mean energy intake per person per day has increased by 100 kcal. (418 kJ) for males and 100 kcal. (418 kJ) for females. The mean intake of protein was 65 g. per day for males and 55 g. per day for females in 1948 to 1949 and 75 g. per day for males and 65 g. per day for females in 1968. The mean intake of fat was 55 g. per day for males and 45 g. per day for females in 1948 to 1949 and 65 g. per day for males and 55 g. per day for females in 1968. The mean intake of carbohydrate was 300 g. per day for males and 280 g. per day for females in 1948 to 1949 and 320 g. per day for males and 300 g. per day for females in 1968. The mean intake of calcium was 1,000 mg. per day for males and 800 mg. per day for females in 1948 to 1949 and 1,200 mg. per day for males and 1,000 mg. per day for females in 1968. The mean intake of riboflavin was 1.5 mg. per day for males and 1.2 mg. per day for females in 1948 to 1949 and 1.8 mg. per day for males and 1.5 mg. per day for females in 1968. The mean intake of vitamins A, B<sub>6</sub> and C was 1,000 IU for males and 800 IU for females in 1948 to 1949 and 1,200 IU for males and 1,000 IU for females in 1968. We can see that the mean intake of all these nutrients has increased by 100 kcal. (418 kJ) for males and 100 kcal. (418 kJ) for females, 10 g. for males and 10 g. for females for protein, 10 g. for males and 10 g. for females for fat, 20 g. for males and 20 g. for females for carbohydrate, 200 mg. for males and 200 mg. for females for calcium, 0.3 mg. for males and 0.3 mg. for females for riboflavin, 200 IU for males and 200 IU for females for vitamins A, B<sub>6</sub> and C.

These differences among the distributions of groups as well as the differences between urban and rural areas are becoming smaller and smaller, but we have still some important nutritional problems, such as a deficiency of iron among females at present. Incidence of regulatory obesity is increasing not only in adults but also in children.

I would like to compare the situation of food supplies and the cause of death by diseases between Western Countries and Japan. The food supply data were taken from the Statistical Yearbook of United Nations, and the data on the causes of death were taken from the WHO World Health Statistics Annals. Compared with the food consumption in Western Countries, the consumption of cereals and pulses are high and that of total calories, sugar, milk, fat and oils is still low in Japan. As for the cause of death, the population is the highest cause of death in Japan, and the death rate caused by this disease is higher than that in Western Countries. On the other hand, the death rates due to coronary heart disease and diabetes mellitus are very low compared with those of Western Countries. However, the incidences of diabetes mellitus, regulatory obesity, gout and dental caries are increasing.



At the end of this paper I shall add a few words about the administrative activities which were carried on after and contributed to the national improvement in Japan. First, I should acknowledge the help given by the Organizations of the United Nations such as UNICEF and the United States and other countries directly after the War. There are four laws enacted after the War in Japan: National Law, National Improvement Law, School Lunch Law and Cocks Law. The number of the training schools for nationalists is 258 and the number of juvenile nationalists is 22,000. As for the school lunch program, the number of the boys and girls receiving the school lunch is 98% of the total primary school children and 83% of the secondary school pupils. As for the encouragement of arts, the rice consumed in Japan is covered with 100% and rebellion. When that bread and needles are very rare, connected with economic rebellion, economic activity. Syrian price is connected with economic. A the one rebellion and economic.

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## A NEW AREA OF BIOCHEMICAL INTERESTS

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Chemistry is one of the most ancient subjects known and has had essentially an applied outlook. It has been instrumental in a number of the widest sense of converting less valuable materials into more valuable ones. It has also been instrumental in discovering the extent to which we could sense that is the discovery and process of the new chemical elements. Consequently its contribution to the progress of the world and hence to the community. This work has been a continuous one and has led to the constitution of modern chemical industries which are now being studied by special methods. However, the teaching of chemistry in the last few years was frequently marked by a lack of interest and a general feeling of difficulty in teaching and understanding of the subject. This has been a very serious problem. Unless lectures are accompanied by highly intelligent and experienced demonstrations and emphasis is laid on understanding of the subject and not be made attractive. In past years, the practical teaching of chemistry seems to have ceased and so has the emphasis on applications of chemistry and consequently it is very clear that the progress of chemistry has suffered during the last century and the early part of this century, is far decreasing.

Though one of the oldest, chemistry is still one of the youngest in vigour and freshness of outlook. It can never become stale or old or exhausted. With more effort and more speed, put into chemical studies, a faster expanding horizon is created with a greater scope in every direction. On a rough estimate, in a ten-year period it gets renovated with a new spirit with new techniques and new areas of study. Consequently the students of chemistry and especially the teachers have to be devoted fully and keep abreast of developments or they will be left behind. They should create the wave and effectively ride it.

Biochemistry as the newest branch of chemistry has become very important in regard to one of the major objectives of chemistry, that is health of human and animal beings. One aspect of it, ascorbic acid (vitamin C), its production and function, was Prof. B. C. Gaha's life interest. A line of study following the discovery of ascorbic acid has in recent years made rapid advance. In the thirties Prof. Nien George, the discoverer of ascorbic acid wrote a small article in *Current Science* entitled "Vitamin C (ascorbic acid)". Therein he narrated how he was led to the discovery of the commonly occurring group of plant





## TOWARD THE CONQUEST OF MALNUTRITION

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I am happy to have this opportunity of paying my tribute to my friend, late Professor B. C. Guha. I had come into close contact with Professor Guha during the years when we were both in the National Advisory Committee—he as the Chairman and I as the Secretary. His death at a relatively young age was a great blow to all of us who were working earnestly in this country.

Professor Guha was not only a true scientist, but also a great humanist. What particularly impressed me was the breadth of his outlook. While Professor Guha reached great heights in the field of nutrition and biochemistry, he also had a keen sense of social responsibility and enabled him to recognise the wide implications of his research in the field of nutrition and the urgent need for action to be taken to meet the needs of his own speciality of biochemistry.

I therefore feel it would be appropriate to dedicate this volume which is dedicated to his memory in a book entitled "Toward the conquest of malnutrition."

During the years his name was mentioned in connection with nutrition and had some significant contributions to the knowledge of public health. This man has been long associated with the Indian Council of Medical Research, and his work has been nearly always in the field of nutrition and the health of our people. He has been a constant reminder to us that we have a long way to go. There has no doubt been considerable improvement in food production in this country, but this has been almost completely offset by the increase in population. The per capita availability of food supply for the last two decades has remained more or less stationary.

### OUR DIET

The figures for per capita availability of food staples are unsatisfactory as they are still well below the minimum requirement in the country. There is considerable malnutrition in the country, and the food intake is different in different regions of the country and in different social classes of our people. The income levels in several groups of our population would hardly permit the achievement of even low-cost balanced diets for the families. Our national



Nearly 25% of our hospital beds, especially in our children's hospitals are taken up by cases of malnutrition. In addition, the present over-crowding of hospital beds and straining of our already overburdened health resources.

And from a purely nutritional point of view, a very tall among women of the child-bearing age. Nutritional deficiency is a major cause of maternal mortality in our country. Our maternal mortality rate today stands at 250 per 1 000 000 livebirths as against 25 in United Kingdom.

Nutritional deficiency diseases like pellagra and goitre which have been eliminated from Western countries where they are today are more than medical curiosities, common to all the vast numbers of our people.

There can be no doubt that the problem of malnutrition poses the greatest challenge to our Government and to our technological education, their ability to meet this challenge would depend the health and the well-being of our future generations.

### MEANS OF PREVENTION

Our strategy in combating malnutrition must include both long-term measures which would eventually lead to an economic improvement of the nutritional status of our people, and short-term measures which would immediately serve to alleviate malnutrition, the weakest and most vulnerable sections of our people. We must now review these approaches.

### IMMEDIATE MEASURES

#### *Specific remedial measures :*

Specific remedial measures are essential to control certain major nutritional deficiency diseases which are widespread in our country today. The three major nutritional problems which fall under this category are the control of rickets in the children, Scurvy malnutrition through a vigorous execution of the programme of administration of ascorbic acid, the control of anaemia in pregnancy through a well-planned programme of distribution of iron and folic acid tablets to pregnant women through the net-work of maternal and child welfare centres in our country, and the prevention of blindness resulting from severe degrees of vitamin A deficiency through an intensive programme of oral administration of massive doses of vitamin A at six monthly intervals to pre-school children in the Southern and Eastern parts of our country. It is gratifying that our Government has provided budgetary allocations for these programmes and it is to be hoped that they will be effectively implemented. Their implementation does not call for any improvement in the economic standards



tively reached in large numbers through schools and where considerable experience with regard to school nutrition programmes is available. On the contrary facilities for reaching pre-school children in large numbers are at a very inadequate and largely unco-ordinated level. The present nutrition programmes among pre-school children have yet to be planned. The immense administrative and financial problems involved in such a programme for pre-school children have up to now not been fully appreciated and understood. In such times as these, these programmes will probably be postponed in spite of improved health care facilities. Our nutrition efforts should be directed towards reaching the pre-school children in the urban areas where there is more measurable help available. This does not mean that the nutrition programme of new towns and of the urban areas of such programmes as existing free dispensaries at the Block level should be extended to include pre-school programmes as an integral part of their health services. Such a programme is already being run in MCH Centres in Pondicherry, Coimbatore, the Madras Mandals and the Bangalore Mandals. It is possible to extend such programmes to some other urban programmes specially designed for this purpose. The success of these programmes will ultimately depend on our ability to harness the existing administrative machinery and the existing framework work at the Block level for this purpose.

It would be desirable if the Planning Commission of the Government of India set-up demonstration-cum-training centres for pre-school feeding programmes for pre-school children in a few selected centres in the country where the requisite cadre and facilities for personnel and evaluation of the programme exist. This will create a body of the necessary expertise for organising and implementing the pre-school child feeding programmes on a country-wide scale.

### *The middle income group :*

The main beneficiaries in most of the nutrition programmes at present envisaged are the poorest segments of the population. A considerable degree of malnutrition also exists among other segments of the middle class — children of teachers, petty shopkeepers, skilled workers, artisans and the so-called white collar workers. The existing pre-school nutrition programmes now being attempted are such that the majority of the population will not be benefited. This is unfortunate because the lower middle classes in many ways constitute the backbone of the economy. While the poorest group should continue to receive attention, programmes designed to improve the lot of the important middle income groups will also lead to progressive divi-

dends. For example, low-cost nutritious biscuits made of inexpensive locally available food grains and oilseeds can be produced in large quantities and form the basis of a nutritious diet for the urban and some rural population. Such biscuits stocked in warehouses and distributed by voluntary agencies can be sold to the needy at nominal price, and the proceeds can be used for relieving the urban segment of the population.

### *Long-term approach*

In the final analysis, the food problem can be solved for our people only through increased food production. The Government has been encouraging food production to a considerable extent, but the rate of increasing population and the increasing demand for food grains and other foodstuffs calls for commensurate steps to be taken. The Government has already decided to be permitted export of all surplus food grains. The Government has also decided to increase food production by 10 per cent in 1965-66. It is hoped that the Government will be able to increase food production by 20 per cent in 1966-67. The Government has also decided to increase food production by 20 per cent in 1967-68. It is hoped that the Government will be able to increase food production by 20 per cent in 1968-69.

Recent descriptions of the food problem in India have been able to meet the food requirements of the population in the near future. If the necessary inputs are forthcoming, we may be able to achieve self-sufficiency in food. However, this approach should not arouse undue expectations and illusions as to the time and the cost involved. The largest of malnutrition implies a lot more than the mere achievement of over self-sufficiency in foodgrains. The existing food and nutritional imbalances which today contribute to malnutrition must be corrected. The achievement of self-sufficiency in foodgrains together with the correction of these imbalances will undoubtedly go a long way in correcting the current widespread chronic shortage, but our diets will still stand in need of considerable qualitative improvement. In fact, our deficiency with regard to foodgrains is relatively of a much smaller order than the gap with regard to other protective food elements like fruits, milk, fish, eggs and meat. Unless these gaps are filled there can be no qualitative improvement in our diets.

It is essential at this point to impart a new national orientation to the so-called agricultural revolution in order to ensure balanced augmentation of food production in accordance with our national needs. New varieties of foodgrains which are released for propagation should be not only high yielding but also of high nutritive value. This is fortunately being increasingly appreciated.



Apart from prestige cereals like wheat and rice, other foodgrains and oil-seeds must receive increasing attention. Millets constitute the staple of the poor segments of our population and demand special consideration. Since foodgrains are likely to be produced in decreasing quantities for some years to come it is essential that the dietaries should maintain the required quantities of pulses and legumes in order to provide proper amino acid balance. The pulse-cereal ratio in the present pattern of food production is already unsatisfactory from the nutritional standpoint and unless high priority is accorded to the promotion of pulse production our diets are likely to become even more unbalanced in future. Now that we are in sight of achieving the national objective of self-sufficiency in foodgrains, the agricultural strategy should be oriented towards the promotion of increased production of other protective food elements like fruits, milk, eggs and meat as well.

Today, fortunately, the importance of nutrition is being appreciated at the highest levels in our country. It is up to all those concerned for the promotion of the welfare of our people to take advantage of this propitious climate and to head the campaigns towards ensuring better nutrition for our Nation.

# ARYLSULPHATASES IN BRAIN

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## INTRODUCTION

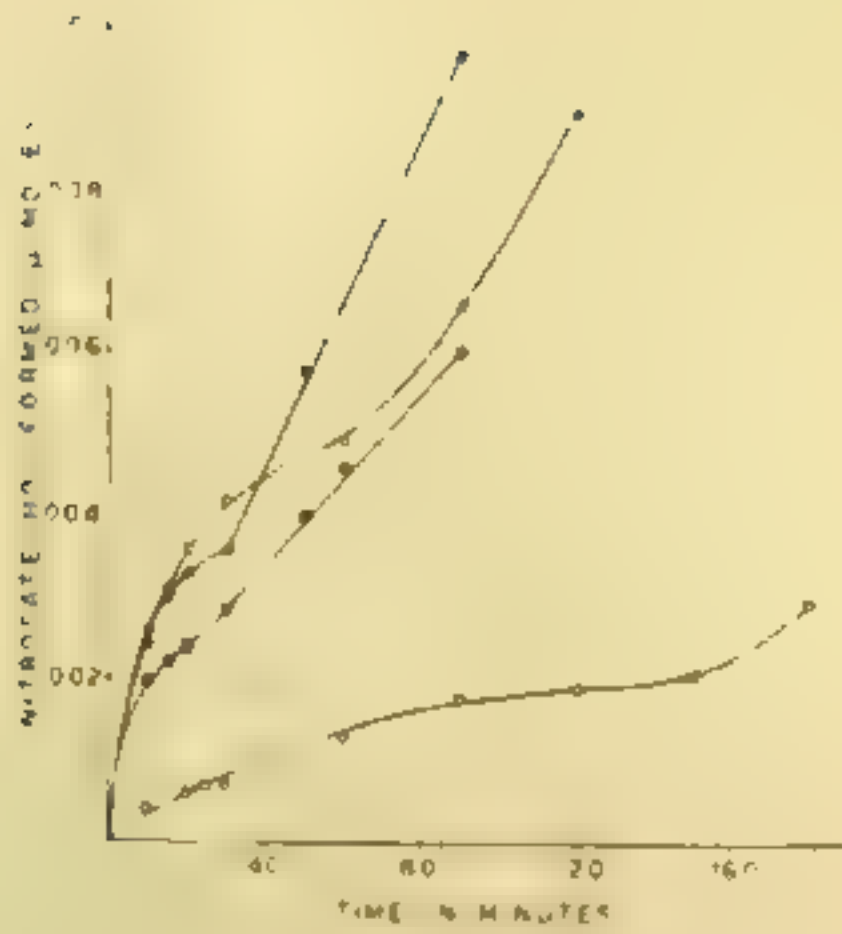
The sulphate ester bond is catalysed by a group of enzymes, the arylsulphatases, with the general reaction:



There are three classes of arylsulphatases, the arylsulphatases, the arylsulphatases and the arylsulphatases. The arylsulphatases are characterized by their high activity towards p-nitrophenyl sulphate and p-nitrophenyl phosphate. They are much less active towards p-nitrophenyl sulphate. They are generally inhibited by cyanide and sulphite but hardly affected by chloride, fluoride, phosphate and sulphate ions. The type I arylsulphatase of mammalian liver, the arylsulphatase C, differ from the corresponding enzymes of most organisms in their extreme instability (4,6). They are located in the microsomes and very difficult to solubilise. The only soluble arylsulphatase C which has been obtained was prepared from rat liver microsomes by treatment with crude pancreatic enzymes in the presence of a non-ionic detergent (4).

The type II arylsulphatases are more active towards simple substrates such as p-nitrophenyl sulphate and p-nitrophenyl phosphate. They are much less active towards p-nitrophenyl sulphate. They are generally inhibited by cyanide and sulphite but hardly affected by chloride, fluoride, phosphate and sulphate ions. The type I arylsulphatase of mammalian liver, the arylsulphatase C, differ from the corresponding enzymes of most organisms in their extreme instability (4,6). They are located in the microsomes and very difficult to solubilise. The only soluble arylsulphatase C which has been obtained was prepared from rat liver microsomes by treatment with crude pancreatic enzymes in the presence of a non-ionic detergent (4).

Type II arylsulphatases have been detected in plants, animal tissues and microorganisms. The most studied examples are the arylsulphatases A and B of mammalian livers (5,6). The type II arylsulphatases are characterised by their high activity towards p-nitrophenyl sulphate and much less activity towards simple substrates. These enzymes are strongly inhibited by sulphite, phosphate, sulphate and fluoride ions but not by cyanide ions. The extraction and purification of arylsulphatases A and B from ox and human brain have been recently described by various workers (7-9).



### *Assay of arylsulphatases A and B :*

There have been difficulties in assaying arylsulphatases A and B when they are present together as in human brain. A solution to this problem has been worked out by Bram, Dodgson and Spencer (13) by utilizing a differential inhibition with chloride ions and obtaining the correct proportions. The determination of arylsulphatase A and B is based upon the following principles :

1. Normal Kinetics are exhibited by arylsulphatase A at low substrate concentrations in the presence of  $2 \times 10^{-4}$  M sodium phosphate.
2. Considerable amount of arylsulphatase B activity under the same conditions.
3. Arylsulphatase B is inhibited by chloride ions when p-nitrophenyl sulphate is used as substrate, whereas arylsulphatase A is not.

The determination of arylsulphatase B in the presence of A is based on the fact that arylsulphatase A exhibits an initial rapid activity for the first 20 minutes of the reaction which is followed by a gradual acceptance in the presence of high molar concentration of chloride ions. Moreover, this reaction may be inhibited by the addition of a small amount of hydroxymercuric acetate which inhibits the activity of arylsulphatase B. As suggested by Dodgson and Spencer (13) this assay procedure of arylsulphatase B is not satisfactory because A and B become the differences in the kinetic properties. However, the difference is so great to enable the independent assay of the enzyme in the presence of other

### *Distribution of arylsulphatases :*

Many studies of the distribution pattern of these enzymes have been made both by conventional and by modern biochemical methods. However many of the results are difficult to interpret because of the failure of investigations to appreciate the complexity of the enzymes. The most reliable information on the distribution of arylsulphatases in mammalian tissues is that of Dodgson and his group for the rat (12) and the human (13). They have shown that in general liver is the organ richest in arylsulphatases and that considerable amounts are also found in kidney, pancreas and adrenal. Arylsulphatase activity in nervous tissue has been reported by Neuberger and Simon (14) in rabbit brain. Arylsulphatase activity in human urine and serum has been reported by Neuberger and Simon (15, 16). The distribution of arylsulphatase in various organs of the rat, guinea pig, rabbit and chick has been reported by Neuberger and Simon (17). In addition arylsulphatase activity has been found in some plants (18) fungi (19) and bacteria (20).

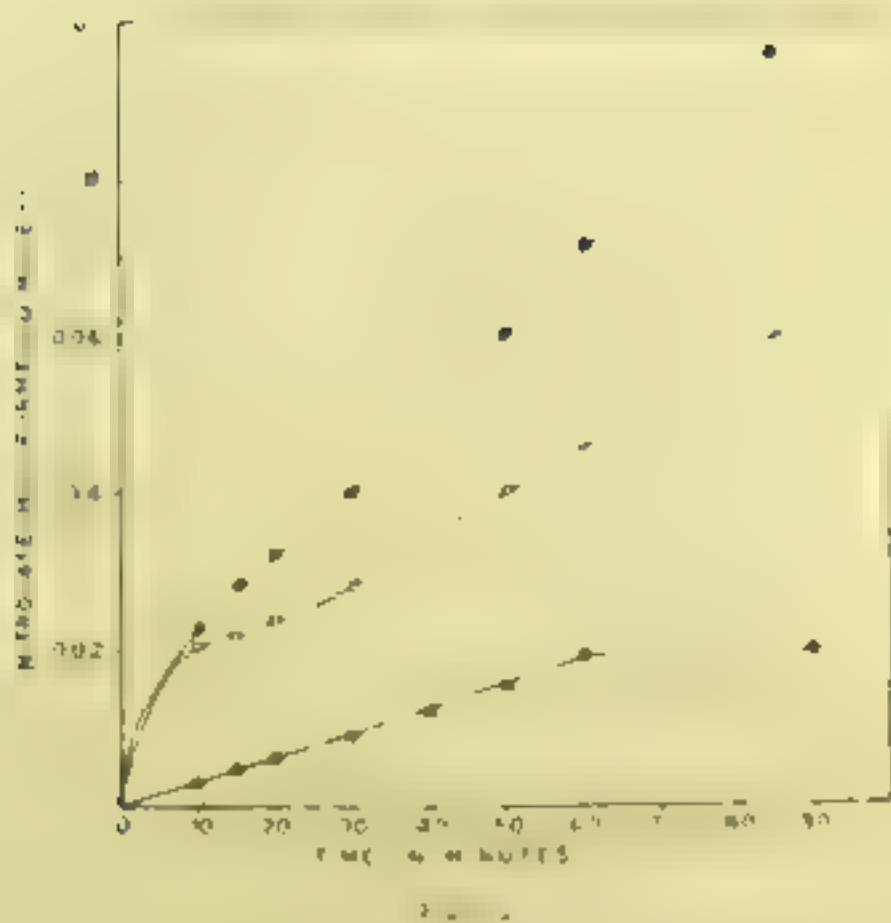
Arylsulphatases have been found in all animal species (21, 22). The quantitative data are available only for man. Hence the systematic determination of arylsulphatase A and B have not been worked out and hence the proportion of these enzymes in each species is not known. Roy (21) has studied the properties of arylsulphatase A and B by separating them by paper electrophoresis and by measuring their activities using p-nitro arylsulphatases A and B as substrates. In a comparative studies in this laboratory (22) on the basis of quantitative assay of A and B in brains of different animal species show that the proportions of these enzymes vary from one species to another (Table 1). In human brain the average ratio of arylsulphatases A : B is 1 : 1. In human placenta and chicken arylsulphatase A accounts for the greater part of the total arylsulphatase activity. In fact in chicken the activity of arylsulphatase A is even more high compared to B. In monkey, rabbit and sheep arylsulphatase A is high while in mammals like rat, monkey and man arylsulphatase B predominates.

Balwahrmanian and Bhattacharya (23) studied the regional distribution of arylsulphatase activity in sheep brain and they found that the activity of arylsulphatases was high in those regions which were rich in white matter. Recently Paronqui and Bhattacharya (24) studied the regional distribution of arylsulphatases A and B in monkey brain (Table 2). The above regional distribution suggest that arylsulphatase A is always high in white matter not only in cerebrum but also in regions like medulla and corpus callosum. The activities of arylsulphatase B are high in grey matter compared to white matter in most of the regions.

Roy (21) studied the intracellular distribution of arylsulphatase in liver and according to him arylsulphatases A and B occur in lysosomes while arylsulphatase C is found exclusively in microsomes. These findings have been recently confirmed by cytochemical staining method (24). Crundenon and Allen (25) studied the subcellular distribution of arylsulphatases in rat brain and they found that the localization of these enzymes was the same as in the liver. Roy (26) has reported that arylsulphatase B of rat liver is localized in the framework of the lysosomes while arylsulphatase A in the sap.

### **Properties of arylsulphatases :**

In general arylsulphatase A is a very acidic protein with an isoelectric point at pH 3.4. The enzyme exists as a monomer of Mr 107,000 at pH 7 but as the pH is lowered towards the acidic side the monomer shows an increasing tendency to associate to produce a tetramer of molecular weight 411,000. This enzyme has high affinity for p-nitrocatechol sulphate (27). The arylsulphatase



D is a highly basic protein and has low affinity for p-nitro-substituted sulphate. More recently studies on arylsulphatase B from ox liver (28), ox brain (8) and rabbit cortex (29) have revealed that the enzyme can be resolved into further fractions by chromatographic or electrophoretic techniques. Aren and Roy (28) have purified and separated two of these forms (B<sub>1</sub> and B<sub>2</sub>) from ox liver. Both have molecular weights of about 25,000 and are kinetically indistinguishable. Under certain conditions of low ionic strength aggregates of these enzymes can occur which are mixtures of polymers with molecular weights of up to 300,000. It is probable that this is probably the main process involved in this aggregative process. A summary of some properties of arylsulphatases A and B is shown in Table 3.

The kinetics of arylsulphatase A are extremely complex because the reaction velocity shows a small dependence with the enzyme concentration and time of incubation (30). The anomalous kinetic of arylsulphatase A is manifested by a modified product formation rate during incubation with p-nitro-substituted substrate, which is a partial recovery of the initial rate. Baum and Dole (31) have observed that during the incubation of enzyme and substrate a new site is formed, which gives rise to a new reaction product. This new site is formed by the action of a modified enzyme, which is activated by p-nitro-substituted substrate. When the substrate is bound to the new site of the modified enzyme, the reaction velocity is low. On the other hand when the substrate is bound to the native enzyme, the reaction velocity is high. When the modified enzyme is bound to the new site, the reaction velocity is low. This hypothesis is recently confirmed by Nishikawa and Roy (32) who actually isolated the so-called "inactive modified enzyme" from incubation mixture. Apparently "inactive modified enzyme" is activated by sulphate but is slightly activated by p-nitro-substituted substrate. Phosphate and oxalophosphate the inhibitors of the native enzyme also activate the "modified enzyme". The low activity of the modified enzyme was found due to a powerful substrate inhibition which is decreased in the presence of sulphate. Furthermore it was postulated that sulphate displaces the equilibrium in favour of the native enzyme primarily through the formation of a native enzyme-substrate complex. The anomalous time-activity curves for arylsulphatase A of different animal species are shown in Figure 1. Arylsulphatase B from different animal species follows normal kinetics (Figure 2).

Recently Faruqi and Richman (33) have purified a unique arylsulphatase A from chicken brain. This enzyme resembled arylsulphatase A of other animal species in its kinetic properties such as  $K_m$  value anomalous

and a strong elavation and inhibitory effect of phosphate, sulphate and sulphate ions. However, the sulphate mobility, behaviour under zinc acetate and sodium dimethylsulphate, Ag<sup>+</sup> and inhibition by citrate ion were similar to arylsulphatase B of the normal person. A similar arylsulphatase A is also recently purified from kangaroo liver by Rex et al. This enzyme shows less pronounced anomalous kinetics, does not polymerize at low pH values, had a different isoelectric point and different  $K_m$  value for sulphate ions compared with ox liver arylsulphatase A.

For chicken brain arylsulphatase A catalyses the desulphation of both p-nitro catechol sulphate and cerebroside 3-sulphate (Figure 3 and 4). The comparison of some kinetic parameters of this enzyme using cerebroside 3-sulphate and p-nitro catechol sulphate is shown in the Table 4. The non-ionic detergent Triton X-100 has the enzyme activity toward both cerebroside 3-sulphate and p-nitro catechol sulphate (4). Like ox kidney cerebroside sulphatase (35) this enzyme has a  $pH$  of 4.5 and almost same  $K_m$  value ( $2 \text{ nM}$ ). Further with increasing 3-sulphate as substrate the enzyme shows a linear increase in  $V$  (sulphate) until about 60  $\mu\text{M}$  and then begins to level off (Figure 5). For p-nitro catechol sulphate the  $V$  increases linearly with substrate and  $K_m$  is about 10  $\mu\text{M}$  (Table 4). Rex et al. (36) have purified a bovine arylsulphatase A using 4-methylumbelliferyl sulphate as substrate. These differences in the kinetic properties of arylsulphatase A may be attributed to the nature of the substrate. It has been suggested that the enzyme which desulphates cerebroside 3-sulphate is different from the enzyme which desulphates p-nitro catechol sulphate. A more detailed comparison made by Merrett and J. J. Henry (38) in the bovine brain has found evidence of two activities.

Tracy et al. (39) and Knecht and his co-workers (36) have indicated that essential amino acid residues of  $K_1$ ,  $K_2$  and other enzymes are glycoproteins and that the amino acid residues of the active site are altered. Recently, Gadjis et al. (40) and Knecht (41) have indicated an approximately 40% of arylsulphatase A and B is composed of carbohydrate. B by neuraminidase treatment and subsequent amino acid analysis produced a similar composition of the two enzymes. Arylsulphatase B. The arylsulphatase B produced by the same source has not increased binding affinity for p-nitro catechol sulphate and cerebroside 3-sulphate. B in biochemical assay. But in the assay using bovine cerebroside sulphate A has more affinity for p-nitro catechol sulphate and cerebroside B has less affinity for this synthetic substrate. It is the basis of the acidic and basic nature of lysosomal hydrolases the above authors have prepared a deficiency of the specific aryl transferase which produces arylsulphatase A deficient in the genetic disorder metachromatic

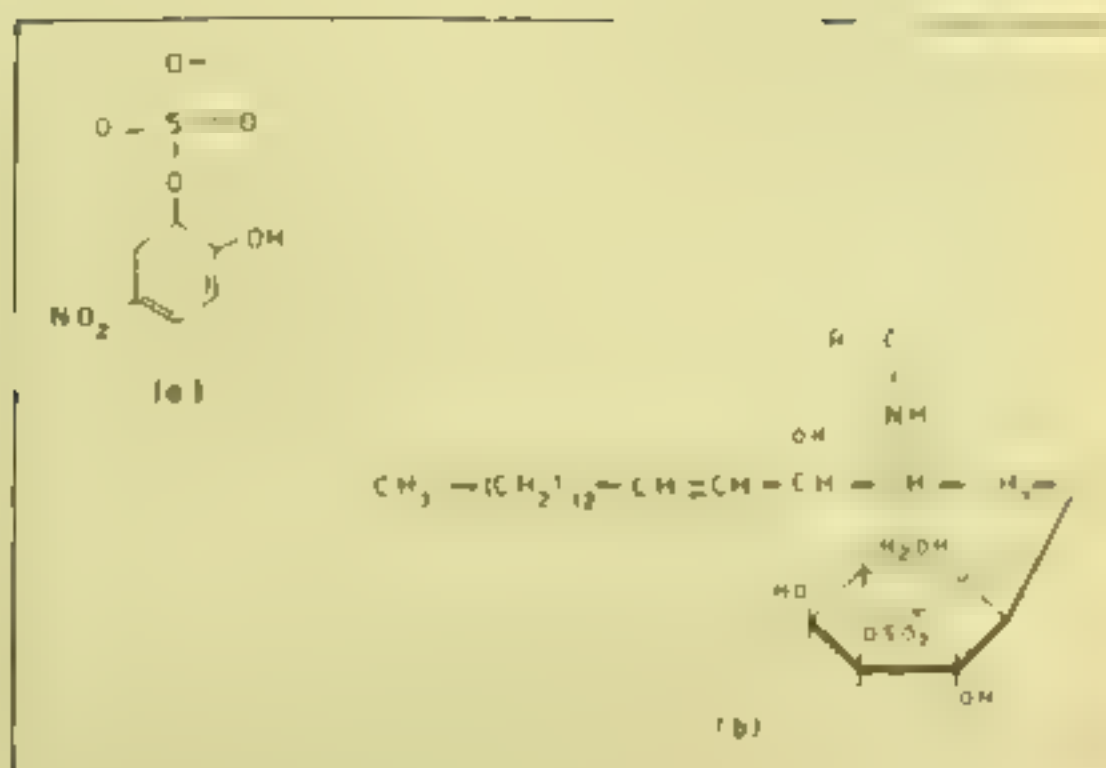


Fig. 3

leukodystrophy, from arylsulphatase B which is present in much smaller amounts in this disease.

### *Role of arylsulphatases:*

Interest in arylsulphatases rose immensely after Armstrong *et al.* (39) using periodate oxidation as a sensitive method for the detection of sulphatase A in cerebrospinal fluid by a direct method. The enzyme was found to be deficient in metachromatic leukodystrophy and was characterized by a very low  $K_m$  for degradation of cerebroside 3-sulphate, brain tissue and cerebrospinal fluid (40) and (41). Figure 67 shows the  $K_m$  and  $V_{max}$  of arylsulphatase A in cerebrospinal fluid by demonstrating that arylsulphatase A is a component of cerebroside sulphatase—an enzyme splitting the cerebroside 3-sulphate. An important interesting aspect in the report of Armstrong *et al.* is the requirement of a water soluble factor which enhances the degradation of cerebroside 3-sulphate 100-fold by arylsulphatase A. Recent work from the laboratory of Jackewitz (42) has indicated that buffer concentration has a marked effect on enzyme activity when cerebroside 3-sulphate was used as substrate. There was an increase in enzyme activity up to a final concentration of 0.1 M but as the buffer concentration was increased to 0.2 M there was almost complete inhibition of enzyme activity. Furthermore, temperature is a variable factor, required only at high buffer concentration (0.2 M) for cerebroside 3-sulphate degradation.

Newell *et al.* (42) have reported that metachromatic leukodystrophy brain extracts contain immuno-reactive proteins which precipitate anti-arylsulphatase A serum. These findings and the data of Porter *et al.* (43) with fibroblasts provide evidence that arylsulphatase A proteins appear to be similar in that they all exhibit extreme lability under normal extraction procedures but they may differ among themselves in the affinity for cerebroside sulphate or in some other parameter which determines latency of clinical onset. The studies by Porter *et al.* (43) and the wide range of reported latencies in clinical manifestations suggest that the metachromatic leukodystrophies result from a broad spectrum of cerebroside sulphatase deficiencies ranging from virtual absence to an appreciable percentage of normal, even in those cases of late onset of symptoms.

The role of arylsulphatase B is poorly understood. However, a marked decrease in arylsulphatase B activity was observed in Hurler's syndrome (44). Further, it was found that although there was more of the sulphatase in the cerebroside sulphate fraction in Hurler's disease compared to normal brain, the mucopolysaccharide content in Hurler's and normal brain was the same. Recently





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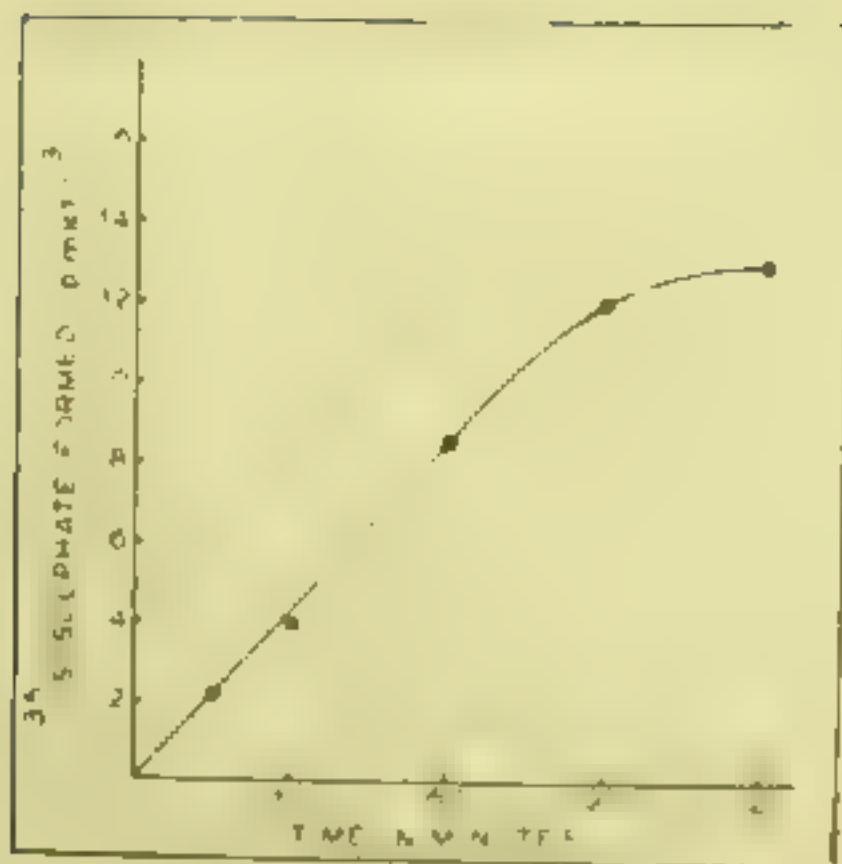
(52) have presented a localization by which multiple enzyme defects can be explained without involving the multiple gene concept. It is tempting to speculate that the multiple enzyme defect may here may reflect a defect in a regulatory gene, a phenomenon which has been observed in bacterial systems but not yet in man (53, 54). A defect in a regulatory gene would imply that the locus controlling these enzymes is a regulatory locus. The precise elucidation of these issues requires more precise information on the structure and function of the various sulphatases and eventually the construction of an accurate genetic map. Murphy *et al.* (51) suggested that the detailed study of patients with multiple enzyme defects may help to resolve these fundamental aspects of human genetics.

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TABLE 1

Ratio of specific activities of Arylsulphatase A and B in brains of various animal species

Species	Specific activity A	Specific activity B	Ratio of specific activity A/B
Rat	0.28	0.58	1/2
Man (adult)	0.1	0.16	1/1.6
Child (2 years)	0.06	0.09	1/1
Hysteria syndrome patient (2 years)	0.13	0.17	1/1.6
Sanfilippo syndrome patient (8 years)	0.02	0.09	1/4.5
Monkey	0.07	0.13	1/2
Sheep	0.08	0.26	2/6
Rabbit	0.08	0.06	1/1
Pigeon	0.07	0.03	2/3
Chicken	0.1	0.003	11/3
Frog	0.07	0.00	1/1

Specific activity is expressed in  $\mu$ moles of p-nitrocatechol formed/mg protein/hr

TABLE 2

Ratio of specific activities of Arylsulphatase A and B in different regions in monkey brain

Region	Specific activity A	Specific activity B	Ratio of specific activity A/B
Cerebrum			
Frontal grey	0.04	0.00	1/1.7
Frontal white	0.00	0.00	1/1
Parietal grey	0.05	0.11	1/2.2
Parietal white	0.05	0.02	1/2.4
Occipital grey	0.05	0.03	1/1.6
Occipital white	0.03	0.00	1/2/1
Cerebellum	0.03	0.05	1/1.3
Quadrigeintal bodies	0.07	0.16	1/2.2
Pons	0.00	0.14	1/1.4
Medulla	0.02	0.19	1/5.1
Corpus callosum	0.00	0.05	1/1/1

Specific activity is expressed in  $\mu$ moles of p-nitrocatechol formed/mg protein/hr

TABLE 3

Differences in properties of Arylsulphatases A and B of rat liver

Properties	Arylsulphatase A	Arylsulphatase B	Reference No.
Electrophoretic mobility	Moves towards Anode	Moves towards cathode	5
Isoelectric point	4.3	4.8	27, 28
pH optimum	4.4-5.2	5.0-6.5	35
Km (mM)	0.4	0.136-0.08 0.19-0.05	15, 28
Effect of inhibition	Inhibited competitively	Inhibited noncompetitively	38
Effect of silver nitrate	Marked inhibition	Slight inhibition	9
Effect of cyanide	No effect	Inhibits	28
Molecular weight	60,000	50,000	28
Effect of increasing the pH	Formal Tetramer	Formal monomer dissociates	27, 28

Table 4

Some kinetic parameters of the chlorine-bromine dehalogenation of  $\alpha$

Parameters	Chlorhydrate 2-magnesium	p-Nitrophenol sulphate
pH optimum	4.5	5
Assay vol.	0.5	0.5
Substrate concn. mg	5	2.5

Table 3

the activity of *Artemisinine* in different organs of patients with the acute & experimental malaria in

[illegible]

ND—Not done

ND - Not done  
 (Note: any of the above is defined as "not done" if a non-integer is indicated in the table)

Table 6

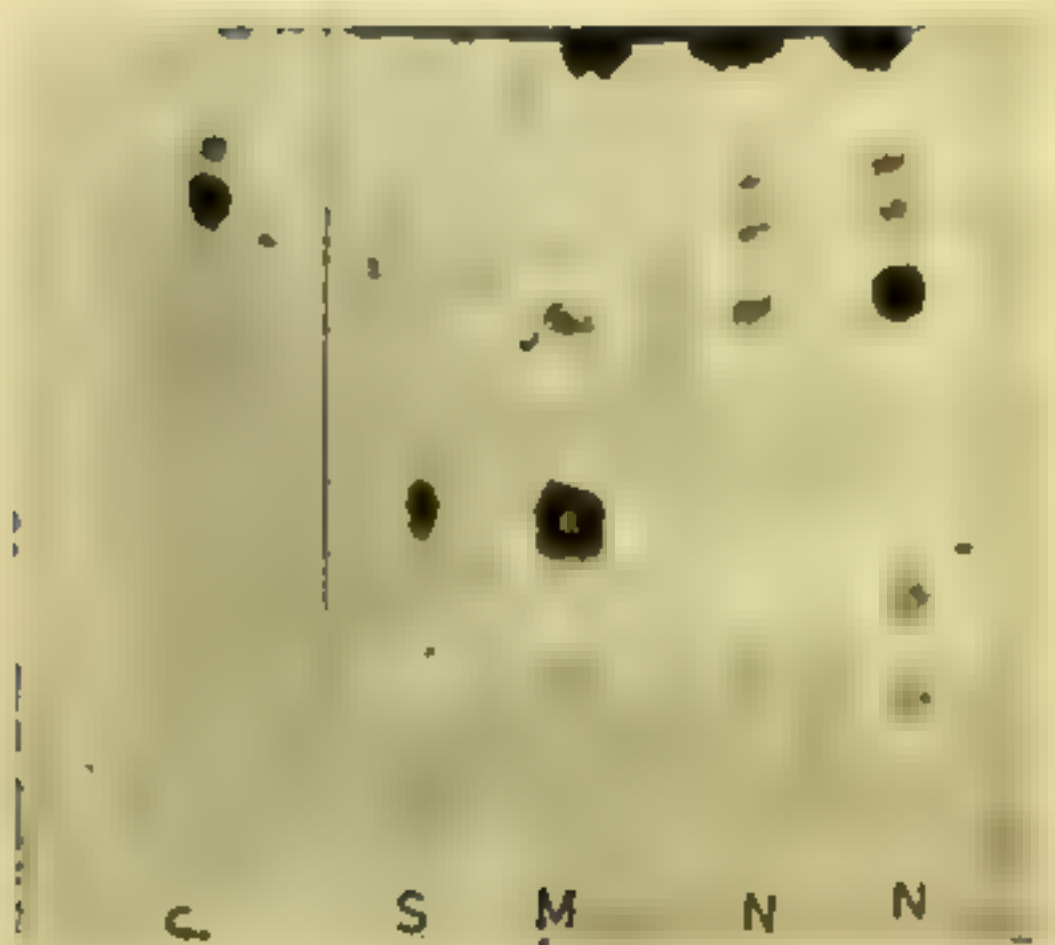
4. With  $A$  and  $B$  as in the previous problem, find  $\lim_{x \rightarrow 0} \frac{A(x)}{B(x)}$ .

Biopsy	Age	Arabinosidase A	Arabinosidase B
Normal	6	7	36.5
Normal	1	10	70.1
Normal	4	4.9	46.3
Normal	1	10	61.4
Normal	1	7.3	40.4
Normal	1	11.3	52.5

Spice in the 19th century was a luxury item for most people.

*E. coli* O157:H7

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1. 1. 1.

## ASCORBIC ACID METABOLISM DURING PREGNANCY AND LACTATION

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It is well known that reproductive performance implies a physiological and nutritional stress on the maternal organism partly because of the physiological changes resulting from the same but mainly because of the parasitic nature of the growing foetus and nursing infant. When the maternal diet is already poor it is likely to become more so with the advent of pregnancy and lactation with the possibility of serious consequences for either the mother or the infant or both. The problem may be acute in developing countries where nutrients such as iron, iodine and thiamine. For instance, iodine deficiency due to maternal deficiency of thiamine and a high mortality due to the same are common in communities consuming polished rice. Cretinism and dwarfism are prevalent in goitre areas. Anaemia is more common in the children of iron deficient mothers (Bhatt *et al.*, 1962). It is also known that the incidence of low birth weight babies, miscarriages, stillbirths and neonatal deaths is higher in communities with a low plane of nutrition (Rajalakshmi, 1971).

Ordinary diets in this country including those consumed by pregnant and lactating women are deficient in most nutrients and the deficiencies are critical in the case of calories, protein, calcium, vitamin A and riboflavin. Although the diets are apparently adequate in iron, iron deficiency anemia is so common. It is not surprising therefore that clinical, biochemical and radiological symptoms associated with deficiencies of these nutrients have been frequently reported in pregnancy. They include anemia, low levels of serum albumin, xerophthalmia and night blindness, osteomalacia, cheilosis and angular stomatitis (Rajalakshmi, 1971).

One would expect the above list to include scurvy or ascorbic acid deficiency as ordinary diets consumed by the poor contain 10-15 mg of vitamin C or less and the fetus at full term contains a store of 70-100 mg of vitamin C. Milk secretion during lactation involves an output of 20-30 mg of the vitamin and to this amount must be added a similar output. Yet scurvy is not always evident in the breastfed infant or the pregnant and nursing mothers and is conspicuous by their absence.

We were alerted to this anomaly more than a decade ago when during the course of investigations on the relation between the composition of diet and

milk with regard to different nutritional aspects, we found the content of vitamin C in milk to be rather low. In the diet Rajalakshmi, Deodhar, and Ramakrishnan (1964) studied, the women showed respectable levels of vitamin C in blood. Nutritional problems were baffled. Several questions seemed to rise including those listed below:

1. Are the common nutritional deficiencies under-represented? Do the women consider vitamin-rich foods of the vitamin such as apple and other common fruits and green leafy vegetables replenished?
2. Are the habits of milk used reliable? Is the concentration of the vitamin in milk increased throughout lactation?
3. Do the levels of vitamin change and build up with the progress of pregnancy and lactation?
4. If not, what are the dietary sources available and required during the period of pregnancy and lactation? Do the women consume C sources?
5. Is it possible to prepare and administer more nutritional tests either naturally or artificially derived, necessary, minor, or other? If so, what value should be assigned to each test?

To get reliable estimates of the vitamin C levels consumed in these families were selected at random from a community health centre to the laboratory and analysed individually. This was done for 23 families for a period of 10 consecutive days. Data were required from the data obtained on the ascorbic acid content of foods and vegetables consumed (Rajalakshmi and Kothari, 1964).

In the studies conducted in the laboratory, 42 subjects in the poor group and 18 subjects in the upper class were studied from early pregnancy till six months post-partum. During the period dietary records were kept of the intake of fruits, vegetables, and other foods including fruits, vegetables, sprouted legumes, etc. A questionnaire of foods consumed by the subjects were collected from the subjects daily, recorded and analysed and the analysed values of foods were with a reference to the previously prepared tables and any missing values were replaced by the average values (Rajalakshmi and Ramakrishnan, 1964).

These studies confirmed our previous impression that the ascorbic acid intake of poor women was rather low. The diet consumed by these families are rather monotonous, consisting of rice and tea in the morning and either roti and dal or vegetable or khichdi and kadhi for the major meals of the day. Vegetables were usually consumed only once a day if at all and the average per capita consumption was about 20 mg. in the form of onions, potatoes, brinjal and bottle gourd. Even the vegetables rich in vitamin C

such as exchange and long-run trends, and that their values, at the time they were entered, the technique of which was the same as that used by Bransby et al (1961) for providing a mean value for the

We also found that food intake dropped during pregnancy. The consumption of seasonal berries and fruits such as the *Vaccinium* species, *Amelanchier* but phlox wood apple etc. are avoided by pregnant women. Although children are allowed to eat them occasionally. The diet is not restricted occasionally by them when in season do not cause any ill-effects on the women.

It must also be pointed out that our figures for rice and grain consumption agree with the 1944 figures for production and availability and with figures given in various diet surveys including the 1945 survey compiled by Mada (1953). In most areas of the country such as Java, Sumatra or Ketula, where the poor class live a diet of rice and some early vegetable consumption may be even less.

[illegible][illegible]

The next question to arise is regarding the probability of our estimates regarding the occurrence of the variation in eye color. In our studies the same was found to be 3 mg. per 100 ml. in our work and was reported to be 1 mg. per 100 ml. by other workers. The values are not in agreement with those obtained in previous studies. The reasons for the difference are not clear and are under investigation. However, the values of Ganesan (1957) and those reported in the book edited by MR. Arakawa, Ganesan and Bhanubramanian (1966).

in these studies, milk samples taken were collected between two feeds. None of these have shown that the low pH and concentration of foremilk and hindmilk supports the relatively high and Mawer (1980) nor does vary with the mother's feed. The milk was collected and analysed



for women, several days such mineral deficiencies are not likely to be detected. In a study conducted by the author, the vitamin C deficiency was detected in a group of women and the diet was supplemented with vitamin C (Dadgar, Rajakrishnan and Ramakrishnan, 1963).

The studies for iron deficiency were also consistent with the results in women (Bajaj, 1958; Rajakrishnan and Kohari, 1964; present author, 1969; and Rajakrishnan, 1971) preschool children (Rajakrishnan and Rajaguru, 1964; Rajaguru and Sankaran, 1967).

The mean daily iron intake of 15-20 mg. of women is comparable with the mean 4 mg. and an average of 10 mg. for men. In the present study subjects were not given iron tablets. It is possible that they obtained for their own iron needs from the diet. In the present study cases, the discrepancy between the iron intake and the iron status of 12 out of 42 subjects was less than 10 mg. per day, which is within the experimental error of the method used for estimation.

The iron status was evaluated by the method of Dill (1947) and was consistent with the clinical picture of iron deficiency symptoms at the onset of pregnancy before the pregnancy was known.

#### *Effect of haemoglobin and haematocrit on iron C<sub>14</sub> excretion*

##### *Delayed appearance of uric acid*

Urine	20-25
Plasma	40-45
Whole blood	80-90
Platelets	120-130*

\*Effect of platelets appears after 112 or 144 hours.

In view of this, it may be expected to occur earlier in the pregnant women, as they were getting iron C<sub>14</sub> from the body stores. In the present study subjects maintained a diet which did not contain iron. A clinical examination by Dr. Bajaj and Dr. Rajakrishnan prior to the study revealed no evidence of peripheral neuropathy, hyperkeratosis, or other symptoms of the interdigital papillae or other symptoms reported by a woman with iron deficiency.

The blood concentration of vitamin C was 0.5 mg. at the onset of 0.5 mg. per 100 ml. of plasma prior to pregnancy and during pregnancy. This value was found to be higher in the pregnant women (0.5 mg. per 100 ml. after 0.5 mg. after parturition and to 0.4 mg. by the end of six months of lactation). Thus women at



the end of six months of lactation had respectable levels of vitamin C in blood whereas the same should have disappeared a long time before these months. Similar observations were made earlier by Bagchi (1965) and on agram by Rajalakshmi *et al* (1965). As a matter of fact, in the studies of Bagchi, the pregnancy values were much higher and no decrease whatever was noted during lactation. In this connection studies in Hyderabad have shown a rise in milk ascorbic acid with the advent of pregnancy in nursing mothers. Some investigators have reported a fall in plasma ascorbic acid during pregnancy (e.g. Teel, Burke and Draper 1938; Bellows and Gumpson 1952). The difference could be possibly due either to a difference in the distribution of ascorbic acid in the plasma and blood cells or due to racial differences. Ascorbic acid is said to be scarce in women  $B_{12}$  (Low-Beer *et al*, 1968).

It must be pointed out that in most of the lactating women, the child is 18-24 months old, the output being about 500 ml or more between 6-12 months and 300 ml or more between 2-6 months.

Thus during each pregnancy and lactation period women must be deemed to lose to the extent 200-300 mg. of ascorbic acid, a more than 500 mg. not to men, in the average of about 5-6 years. The depletion of ascorbic acid of these losses during the lactation period should be made up by the support either from the diet or from exogenous sources. It is interesting to note that many of these women become pregnant again before the previous child is weaned and continue to do so well into the next pregnancy. For example, ascorbic acid was not found to decrease with lactation period. In this connection Stepanow (1958) found no differences in blood vitamin C between women with children and those without.

To ascertain the extent of ascorbic depletion, if any, women at term after partum and at six months of lactation as well as non-pregnant and non-lactating women were given acid tests of the vitamin (40 mg. per day for 5 days). Again no differences were found between the different groups. Essentially similar results were obtained by Bagchi (1965) and in previous studies in this laboratory (Rajalakshmi *et al* 1965).

It must also be pointed out that while not many investigators have set out to study ascorbic acid balance, from the evidence a similar negative balance is to be inferred from several observations. For instance, Pothuk (1958) reports that lactation is successfully maintained by Indian women on a diet containing less than 1 mg. of ascorbic acid per day. Even at an estimated milk yield of about 400-500 ml with an ascorbic acid concentration of about 2-3 mg. per 100 ml, and indeed without any lactation performance at all, the

data would need explanation. The subjects in this study are reported to have shown no evidence of any deficiency symptoms.

Butcher *et al.* (1963) have reported that nursing mothers subsisting on a diet of bread and butter in the North of England, with reported plasma levels of  $1.5 \mu\text{g}/100 \text{ ml}$  of plasma, had tests, plasma level of the vitamin, and a low level of the vitamin in the milk. A similar finding was reported by a group of investigators (1963) who reported that a well-nourished upper-class woman in the post-war period had a plasma level as well as a milk level of the vitamin in the milk.

In all these studies the subjects had no diet low in the vitamin. In the study of Butcher *et al.* (1963) the level of the vitamin (20-30 mg) in milk was found to be lower than in the plasma of the infant than in that of the mother.

It is worth noting that the levels of the vitamin in milk during the war were found to be lower than in the post-war period (20-30 mg/100 ml) were found to be lower than in the post-war period (Tennant, Stearns and McCy, 1950).

Other similar cases have been reported by Butcher (1958) in his report for which full bibliography is given in the appendix.

Even apart from the above, there is a serious concern over the fact that women living in this country provide no more than about 100 g of vegetables and 150 g of fruit in their diet. This provides about 700 ml of milk containing 2 mg of the vitamin C per 100 ml of milk. There is also a serious concern about the fact that vitamin C is not found in breast fed infants or nursing mothers.

It must also be noted that this has been in Europe, while it may be the position with regard to the diet in the rest of the world at the turn of the century or even a few years after World War I, most of the population in the urban areas of Europe and North America lived on a diet of bread and butter. The nursing mothers in these families cannot be expected to have derived much more vitamin C from their diet than I did in a similar diet. Even in a relatively recent study on nursing mothers (Brashby *et al.*, 1964) a considerable proportion of the subjects were found to have plasma levels of the vitamin C of 25 mg of vitamin C. These women may have been receiving at least this amount of vitamin C. It is indeed remarkable that a single case of deficiency in a nursing mother appears to have been reported in the medical and nutritional literature.

In summary, nursing mothers in many areas of the world may be a group in whom the supply of vitamin C is precarious as compared to the requirement of lactation. At least a small proportion of them can be expected to have

starvy or subnormal vitamin C deficiency and yet this has not been found to be the case.

The colorimetric method of Roe and Kuehner (1943) was used in these studies for the estimation of ascorbic acid. A question may be raised regarding the reliability of the method. This method measures both ascorbic acid and its derivative 2,3-diketolascorbic acid and cannot therefore result in an over-estimation of the net amount of the vitamin lost in milk and urine. On the other hand, there could have been an over-estimation of the physiologically potent amount derived from foods. A further check would be the direct assay of ascorbic acid in the milk and urine.

The only hypothesis that would account for the observed reduction of synthesis of ascorbic acid in a host of normally well-nourished women pregnant and/or lactating. The following workers have examined the subject by Division and Piskin (1952), and also McColl (1953), and others (Baker, Beriman and Prough 1955). However, the hypothesis that the reduction with the incidence of scurvy in human beings is due to a deficiency of ascorbic acid with vitamin C derivatives as a result of a poor diet is not in general accepted. It is not sufficient to maintain the synthesis of the vitamin. Baker *et al* (1955) have found evidence of ascorbic acid in the urine of women taking D-glucuronolactone.

The possibility of placental synthesis of ascorbic acid has been suggested by Bagchi (1958) who found no difference in the placental concentration of the vitamin between those who were complicated with ascorbic acid and those who were not. Histochemical studies carried out by the same author using silver nitrate showed the presence and a normal distribution of ascorbic acid in the placenta. It is known that the placenta is not only a transmitting organ but also a highly active metabolic site capable of synthesizing highly complex compounds such as hormones and enzymes. According to Bagchi, the high concentration of ascorbic acid in the placenta cannot be due to storage as no such function has been attributed to it except for some reserves of glycogen and fat. There is not a similar high concentration of other nutrients which are transmitted generously to the fetus (e.g. calcium and iron). Nor is its structure suitable for this function.

In studies carried out in this laboratory, human placenta was found to contain the intermediates and enzymes of glucose metabolism. The former included pyruvic acid. Its oxygen uptake in vitro was quite high (170-190 ml per g per hour). Another interesting observation was the distribution of ascorbic acid and its derivatives in the placenta and in the









in the brain, which will be discussed in detail in a subsequent paper (Gallot, Dahl and Elliott, 1965).

As a consequence of the above, it is not surprising that the level of glutamate decarboxylase (GAD) is higher in the brain of GABA-deficient mice than in the brain of normal mice (Gallot, Dahl and Elliott, 1965). A further indication of the role of GABA in the brain is the fact that GABA may act as a neurotransmitter in the brain (Gallot, Dahl and Elliott, 1965). The fact that GABA is a neurotransmitter in the brain is supported by the fact that GABA is released from the brain (Gallot, Dahl and Elliott, 1965).

It is not yet clear whether the above results are an indication of a deficiency of GABA in the brain or of a deficiency of GABA in the brain. We are now trying to determine whether the above results are due to a deficiency of GABA in the brain or to a deficiency of GABA in the brain. The above results are consistent with the results of the research which has been carried out by the group of Magoun (1962). The above results are consistent with the results of the research which has been carried out by the group of Magoun (1962). The above results are consistent with the results of the research which has been carried out by the group of Magoun (1962).

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calories and vitamin A. Sugars were also present in the diet of a diet poor in protein quality and of minerals and vitamins.

Additional findings showed that the deficiency of protein deficiency on the growth rate of the fish was not significantly different from the control group. The deficiency of protein on the growth rate of the fish was not significantly different from the control group.

**lysis and respiration**

In all the experiments the animals were kept on a standard laboratory diet (Purina 5050) and water *ad libitum*. The animals were kept in a temperature-controlled environment (22°C) and the light and vitamin mixtures were used.

### Protein 2

Postcard rates were fixed at 20% of the current first-class rate, but letter rates were not. In 1961, the postal rate was 10¢, but letter rates were 13¢, as the cost of a 3X4.5" postcard was 13¢ (see Runkelmann 1990). The postal authorities were not aware of the fact that the low postal rates could be used to attract mail from the U.S. (as happened with the low postal rates of the United Kingdom, see Runkelmann unpublished).

The model that may be used to describe the effect of the treatment with protein kinase C activator on the release of  $^{45}\text{Ca}$  from the cells is that the activator produces a decrease in the intracellular  $\text{Ca}^{2+}$  concentration. The decrease would produce a shift of the  $\text{Ca}^{2+}$  release from the  $\text{Ca}^{2+}$  stores from the  $\text{Ca}^{2+}$  release (Kahn 1984). Using this model, the  $\text{Ca}^{2+}$  release is expected to change levels of glutamic acid and GABA.

As ordinary diets in poor countries are deficient not only in quantity but also in quality, studies were made of the effects of improving protein quality on these brain enzymes. Supplementation of kedri (Rajalakshmi Patel and Ramakrishnan, 1969) a milk containing 8.3% protein and is deficient in lysine that is, only 0.15% of the total amino acids, was found to increase the levels of GADH and GAD. A similar result was obtained with addition of lysine rich soyabean meal to a milk powder based diet in rats (Patel, 1970).

Similar comparisons of the amino acid sequences of the 6<sup>+</sup> and 6<sup>-</sup> proteins were made. The 6<sup>+</sup> protein was found to be more similar to the 6<sup>-</sup> protein than to the 6<sup>+</sup> protein.



As the original experiments on protein content were done with 5% and 20% protein and an increase in body enzymes was obtained by supplementation of a diet containing only 7% protein without increase in nitrogen content, a question arises as to the critical level of protein needed to prevent the effects of protein deficiency on these enzymes. In a subsequent series of investigations protein content was varied at 5, 6, 7, 8, 10, 15, and 20%. With a protein content of 8% or more, no definite decrease in enzyme activity was found (Rajalakshmi, Parameswaran, and Ranjithkumar, unpublished). When less than per cent these were only slight whereas deficiency was observed with six per cent protein (Table I). The lack of hepatomegaly and decrease in GAD and GAD should have been expected with the more marked deficiency. The absence of such deficiencies goes to show that a higher protein content and perhaps still higher concentration of individual amino acids such as phenylalanine may compensate for their poor protein quality.

Since the amount of protein needed for inhibition of protein deficient animals may be more than that needed for preventing the effects of deficiency, in rats fed a 5% protein diet, it was observed that when fed with 8, 10, 15, and 20% protein diets, the enzyme activities were returned to normal levels with 15% or more protein (in diet). Inhibition was only partial with 8% protein (in the form of casein).

The question arises from the above experiments whether decreasing the protein content below 5% would prevent the effects of the deficiency. Dietary protein content was varied at 0, 1, 2, 3, 4, 5, and 20 per cent (Rajalakshmi, Parameswaran, and Ranjithkumar, unpublished). At levels below 4% there was definite weight loss. With 4% protein and about the same percentage of protein (calories/body weights were not maintained). This is consistent with the observation that about 4 per cent protein calories are adequate for maintenance but not for growth (Miller and Payne, 1961). Decreasing protein levels below 5% was not found to decrease further GAD activity.

A most surprising observation was made when the protein content of the diet was reduced to nil or negligible amounts (0.2 per cent in the starch source used). Although, as expected, the animals lost weight and died if not killed earlier, the decrease in enzyme activity did not seem to be reversed. The remarkable results were confirmed by repeated experimentation and would need a satisfactory explanation. They certainly suggest that the effect of complete protein deprivation may not always be the same as that of a low protein diet. The results are however consistent with the fact that the animals in these groups were living weight and tissue catabolism might be expected to result in the release of hormones for the removal of which normal level of

glutamate deficiency may be important. In this connection it has been reported that during chronic protein deprivation the glutamate increases (Lehr and Gayet, 1966).

State was also varied in the study, the duration of deficiency needed for the production of motor deficits. Groups of animals were kept at 1, 2, 3, 4 and 5 weeks of protein deprivation. The motor deficits observed in glutamate deficiency were observed only after 5 weeks of protein deficiency and were found to be absent at 3 weeks (Ramaswami, Ramakrishnan and Ramakrishnan, unpublished).

The above experiments were repeated at a lower protein level. It is commonly believed that the extent of protein deprivation is directly proportional to the experimental results obtained. In the present study, when the protein level was 12% of the normal level, the motor deficits were observed after 12 weeks of deficiency and were absent after 6 weeks. The ability to detect effects of deficiency was found to be directly proportional to the duration of deficiency. The motor deficits were found even when the protein level was 10% of the normal level. It is concluded that motor deficits are not expected to be observed unless needed for a long period of time. A long period of deficiency would affect the older animal.

In this connection it may be recalled that as protein deficiency progresses vitamin deficiencies are associated with CNS disorders. In contrast to the above (West and Todd, 1966) Minkowski and his colleagues (1960) reported that a protein deficient rat with a variety of deficiencies including the deficiency of B<sub>12</sub> (Wake, Blomach and Spector, 1958).

### *Glutamic acid :*

Several beneficial effects have been claimed for glutamic acid supplementation on the psychological performance in children (Zimmerman, Bargemeister and Putnam, 1949) and experimentally animals (Zimmerman and Ross, 1944).

Supplementation with glutamic acid was found to reverse the effects of a low protein diet and restore glutamate concentration to normal level (Ramaswami, Prasad and Ramakrishnan, 1964) whereas supplementation to a high protein diet had no effect (Ramaswami, Prasad and Ramakrishnan, unpublished). The observation regarding the beneficial effect of glutamic acid needs explanation. It is understood that it is known that the blood brain barrier at birth may do so at a very slow rate. At a later age, it is known that the blood brain barrier against the passage of substances by means of physical or chemical methods such as dialysis is not effective. The blood

(Purpura *et al.*, 1950). It was suggested that the LF diet may bring about changes in the composition of the body tissue. This suggestion in some instances is supported by the fact that the body composition of mice fed LF diets may be different from that of mice fed normal diets (Sawyer and Stewart, 1960). Some authors have suggested that the body composition of the obese rat is similar to that of the LF diet rat (Purpura *et al.*, 1950). The observation that the body composition of the obese rat is similar to that of the LF diet rat is not necessarily true. The body composition of the obese rat is similar to that of the LF diet rat only if the restriction is severe. In a study by Ramakrishna (1961), the body composition of the obese rat was found to be similar to that of the LF diet rat only if the restriction was severe. In a study by Ramakrishna (1961), the body composition of the obese rat was found to be similar to that of the LF diet rat only if the restriction was severe.

The study of the effect of protein restriction on the growth of a mouse and of the effect of protein restriction on the growth of a mouse (Purpura *et al.*, 1950). This study showed that the growth of a mouse fed a protein restricted diet was slower than that of a mouse fed a normal diet, although their protein quality is poor.

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**Vitamin A :**

Studies were made on the effect of protein deficiency on low and high protein diet. A dose response relationship was observed in the activities of both GDH and GAD (de Vries, 1970; de Vries and Pijl, 1971; unpublished).

It has been reported that the brain is not able to store vitamin A. It remains to be investigated whether the brain could be fed with diets containing adequate amounts of this vitamin. The brain is rich in vitamins needed for pyruvate transamination and gluconeogenesis.

**Maternal deficiency :**

Maternal protein deficiency during pregnancy has been shown to have profound effects on the development of the foetus and on the diet behaviour of the young. It was found that the young of protein deficient mothers were smaller and heavier than those of protein adequate mothers (Khan and Ullrich, 1969; and Khan, 1970). The results are not surprising as the young of protein deficient mothers are born with a low energy reserve and have a low energy reserve when born. However, even when the young of protein deficient mothers are fed during the neonatal period, a significant behavioural difference is observed.

**NAD glycohydrolase :**

There is a significant correlation between NAD levels and (Rajalakshmi, Raj, Thirakumar, Thirumangalakudi and Pankaj, 1971; unpublished).

**Psychological performance :**

Most of the studies on the effect of protein deficiency on the psychological performance of the brain have been done in rats. It is known that the adult brain in Man is not able to synthesize new proteins, but it is able to synthesize new proteins in the neonatal period and in the period of rapid growth.

**Regional differences :**

As the brain is a highly specialized organ, it is not surprising that studies on protein deficiency were limited to the whole brain. As a result different regions of the brain show different effects of protein deficiency and metabolic activity (Rajalakshmi, Thirakumar and Ramesh Kumar, 1971; unpublished). But it is interesting to note that this does not alter in their susceptibility to the effects of protein deficiency.

**Oxygen consumption under different conditions :**

The oxygen consumption of brain tissue slices was found to be influenced by protein deficiency. It may be due to the fact that the brain is rich in the IP amino acids, glucose and amino acids. This difference was decreased or reversed with the use of a specific inhibitor (Rajalakshmi, Thirakumar and Ramesh Kumar, 1971).



- [illegible]

## THE PROTEIN NUTRITION PROBLEM

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### INTRODUCTION

Protein deficiency is a common occurrence in a large portion of the World's population. Over 30 million children suffer grossly from retarded physical growth and development because of lack of right food and deficiency of protein. Protein deficiency is rampant in the developing countries but substantial deficiency is not prevalent. According to FAO's estimate in 1956, only 1 per cent of the population of the world is in a state of shortage of protein, the majority being under 5 years of age.

Although protein deficiency may produce severe deficiency along with protein deficiency known as Protein-Calorie Malnutrition (PCM) compatible with kwashiorkor. The deficiency is not always fatal but it impairs growth and development of the growing child. Apart from the effect on growth and development, protein deficiency is a serious group in the developing countries. It is a major cause of mortality and morbidity and is a leading cause of death in children under 5 years of age. The mortality of such diseases caused is 20-30 per cent in children under 4 years of age groups.

Pregnant and lactating mothers form another vulnerable group in whom the low protein intake may manifest in many ways. There is some evidence that a relationship exists between low birth weight and low protein intake of mothers. Pregnancy is followed by lactation which involves increased loss of protein from the body and consequently an additional protein requirement. Due to the established fact that human milk and animal milk components of milk from malnourished mothers are significantly different from the milk from well nourished mothers, the protein need for milk secretion must be derived from mother's own tissues and consequently from her dietaries.

In contrast to young children and pregnant adults show a marginal deficiency which is sometimes seasonal and sometimes complicated by chronic diseases.

### PROTEIN SUPPLIES AND DISTRIBUTION

Two sources of information are drawn upon to assess the current protein situation. The first one is the consumption of Food Balance Sheets (FBS) by FAO covering 90 per cent of World population and the second is the family or household food consumption survey. FBS shows average available

supplies of calories, protein and fat per capita per day in the country for the particular year derived from the production, imports, exports and disposal of stocks. The available areas for crop production are calculated in terms of calories, protein and other nutrients for each sub-region of different food groups, and for socio-economic groups and for natural ecological regions of the country.

The estimated supplies of protein per capita per day are in grammes per capita per day in India and in countries of the world are given in Table I.

TABLE I

*Energy and Protein Supplies (1961-65) per capita per day*

Regions Sub-Regions		Calories	Animal Protein	Vegetable Protein	Total Protein
Far East	South Asia	2150	4	1	54.8
	East Asia	2350	20.5	1	75.1
	China	2150	1	1	55.0
	China (mainland)	2150	1	1	55.0
Near and Middle East		2150	1	1	55.0
Africa		2150	1	1	55.0
East South Africa		2150	1	1	55.0
Latin America		2150	1	1	55.0
Developing Regions		2150	1	1	55.0
Europe incl. U.S.S.R.		2150	1	1	55.0
North America		2150	1	1	55.0
Oceania		2150	1	1	55.0
World		2150	1	1	55.0

In Table 2 are given the percentage contribution of different food groups to total protein supplies.

TABLE 2

*Percentage contribution of various sources to protein supply*

Regions Sub-Regions		Cereals	Pulses Legumes	Vegetable sources	Meat Fish Eggs	Milk	Animal sources
Far East	South Asia	49	15.0	34.1	9	1.8	15.7
	East Asia	45.7	2.6	34.5	17.6	2.1	27.5
	China	45.1	27.0	55.0	2.0	10.0	12.0
	China (mainland)	37.8	20.3	66.1	17.2	0.5	13.9
Near Middle East		67.8	6.7	20.4	10.1	9.5	19.6
Africa		54.7	15.7	16.4	9.8	4.3	16.6
East South Africa		55.1	15.6	76.9	16.5	6.6	23.1
Latin America		39.8	16.4	64.3	22.9	12.9	25.7
Developing Regions		37.3	16.8	61.4	12.9	9.4	18.6
Europe incl. U.S.S.R.		36.3	9.8	51.5	4	18.8	48.3
North America		17.6	4.6	1	1	24.9	69.9
Oceania		24.9	2.4	1	4.1	22.5	66.9
World		47.9	12.1	68.2	20.7	10.9	31.1

It is observed that in the developing countries the daily per capita protein intake varies to a great extent. The average is 57 gms. (the range 50-75 gms) in the developed countries. South-East Asia is barely over 40 gms. The average protein intake for people from animal sources 40-60 gms/day is just about sufficient to meet the average requirement of 60-70 gms/day. The average protein intake is 57% (68% in the Near and Middle East) of the minimum requirement of protein from animal products and does not supply the protein quality as understood in the developing countries.

The problem of protein deficiency is assessed by questionnaire and questionnaire. The protein deficiency on the rat factor,  $\alpha$ -amino acid (DC) which measures the percentage of absorbed nitrogen of the dietary protein and the Biological Value (BV) which measures the efficiency of absorption and measures the absorbed nitrogen that is retained in the body. The product of these two factors is the protein efficiency ratio (PER) of food consumed. This is related to the body protein content. NPU is another dietary protein index which is related to the efficiency of absorption of dietary protein. The ratio NPU/per cent NPU is related with the retention of protein in the body. In such cases, a value of 1.0 is a further step in the protein efficiency of diet. Efficiency of absorption may be combined in a single index which has been called the protein efficiency ratio (PER) as found by Pridmore and Mendenhall. The deficiency is obtained by multiplying protein concentration by NPU and comparing the result with level

### PROTEIN REQUIREMENT

The protein requirements are given in Table 1 according to the recommendations of Expert Group of FAO and WHO on 'Protein Requirements' depending on body weight and population breakdown according to age and sex. These protein requirements are stated in terms of 'hypothetical' 'Reference Protein' (NPU 100) and then expressed in terms of 'Local Protein' taking into account the average NPU of local protein (NPU) of the country in question. FAO has determined the NPU of the local protein in India which is 53%. The protein requirement is shown in Table 2.

If we compare the protein requirement with the protein supplies of the population, most of the protein requirements or the supplies and the requirements are marginal. The requirements of about 40% of the population of the country are not covered by the supplies. The protein requirement of the great majority of the population (90-95%) has been proposed by the Joint Expert Group\* has the average supplies of 10% of the

mean requirement. This is considered as *Protein 4—me\** or the Recommended Allowance on the basis of which the production prospects and food policy should be based (Table 4).

Furthermore, to fulfil the quantitative aspect of protein requirement the calories derived from dietary protein should be equal to above 1% of the available total supplies of protein (Table 4).

TABLE 3  
*Protein requirement*  
Gm per Kg. body weight per day

Age (months/years)	Recommended Practical Allowance	Energy Protein Ratio (Kcal/Gm)	Total Protein
Infants			
3-6 months			1.8
6-9 months	1.5	1.5:0.91	2.5
9-12 months	1.2	1.2:0.53	2.1
Children			
1-3 years	1.06	1.06:0.53	2.06
4-6 years	0.97	0.97:0.53	1.85
7-9 years	0.92	0.92:0.53	1.71
10-12 years	0.96	0.96:0.53	1.62
Adolescents			
13-15 years	0.84	0.84:0.53	1.58
16-19 years	0.77	0.77:0.53	1.45
Adults			
All Ages	0.71	0.71:0.53	1.34

Example \* Dietary protein requirement of Indian adult

65 Kg man—87.5 gm  
65 Kg woman—65 gm

TABLE 4  
*Available food protein and protein requirements per unit per day*

Regions/Sub-Regions	Supplies		Requirement		Protein Calories	
	Calories	Total protein	Calories	Total protein		
				Mean	Pract.	
Far East + South Asia	2040	54.8	2250	45.0	98.0	11.5
Far East	1500	40.0	1650	33.0	68.0	11.8
South Asia	540	14.8	600	12.0	30.0	11.0
China (mainland)	100	2.0	100	2.0	5.0	8.0
Near Middle East	2400	73.6	2400	45.0	77.0	11.8
Africa	1500	40.0	1500	30.0	60.0	11.9
Latin America	2500	60.8	2500	45.0	85.0	11.5
Developing Regions	2400	57.6	2250	45.0	81.0	11.8
Europe (incl. USSR)	3000	87.6	2600	50.0	88.0	11.4
North America	2400	63.1	2430	61.0	93.0	11.3
Oceania	1,400	40.4	2,000	46.0	90.0	11.5
World	2360	66.1	2370	51.0	66.0	11.1

## PROTEIN GAP

The following table shows the marked difference in the supply and the requirement of protein:

[illegible]

When the Commission's concern  
is not to protect the interests of  
the public, but to protect the interests of  
the private, it is not a public body  
and its actions are not binding on  
the public.

1. The first step is to identify the problem or question that needs to be answered. This involves understanding the context and the specific requirements of the task.

prolonged periods of time, the growth of the population grows slowly.

He has a very strong sense of duty and is very loyal to his country. He is a very good person and is very kind to his friends. He is a very good person and is very kind to his friends. He is a very good person and is very kind to his friends.

1. The first step is to identify the problem or goal. This involves understanding the current situation and what needs to be achieved.

For the above, the protein is distributed widely in different regions of the cell, and is often found in the membranes of the same family.

• If  $b_1 \neq 0$ , then  $\mathbf{b}$  is not orthogonal to  $\mathbf{a}$ .

the lack of a variety of more "exotic" foods in the diet, means that many individuals are at risk of deficiency of key vitamins and minerals. Animal products are a good source of

(b) In general, the size of the program is not too

*W. G. F. I = T A S. P. 2*

Take up 1965 as base year the food production to rise up 1975 to 1985 should increase 74 times to keep pace with the population growth 70% of which will account for the population growth and 4% for increased income. Techno

cally the policy is feasible. It is a national responsibility to make the essential food items available to all, even if it means that some people or rich countries should have to pay more for them. It is not the responsibility of the rich cash-rich countries to develop the food production in the poor countries.

The question whether we should have a free world market for food is a matter for the developing countries to decide. The developed countries like the North Atlantic Treaty Organisation and New Zealand. It is the official view of FAO that the developing countries should first of all satisfy the primary staple food requirements of their own people and then need for rapid increase in food production in the developing countries.

In 1962 food import cost of the developing countries was \$1.5 billion. If increased demand for food is to be met, it would be \$40 billion by 1985. Secondly, it is a common principle that the developed countries should be agreeable to provide food to a poor country if it is unable to produce agricultural products. The people must find employment in the countries where they produce.

#### PROJECTION INTO 1975 AND 1985 FOR INDIA

It is necessary to see whether the food requirements are met or not. The Long Range World Plan of FAO, 1965, was the first and adopted 1985 as the horizon year for the developing countries. The first objective taken into account in the report was to find out the gap between the production goals of the world and the food requirements of the world by country. The case of India is presented here.

Considering the population growth projected into the year 1985 using the median variant of the United Nations medium variant, it is found that 80 to 90 per cent higher protein supplies will be necessary by 1985. Obviously, that fraction of the population whose requirements are already covered will grow in number and its consumption will continue to increase in both better quality. Assuming for this segment a 2 per cent increase per year and elasticity of protein demand of 1.3 (1.05 for the case of India) the increase in protein supplies will have to be 10 per cent by 1975 and by 75 per cent in 1985.

Table 5 presents the values of protein supplies (increase in production of protein foods) required by 1975 and 1985 to cover the extra requirements. It is defined as 20% of Mean requirement (M). The Table also gives indices of production of foods required to attain the objective (100% of the supplies



by the same dates, as compared to the supply available in the base year of 1965.

Table 9

Estimated protein supply and demand in developing countries

Developing Regions		Estimated protein supply (kg)		Estimated protein demand (kg)	
		1975	1985	1975	1985
Far East	South Asia	131	167	159	192
	East Asia	119	133	119	133
	India	151	191	161	203
	China (mainland)	126	151	147	174
	Near Middle East	131	172	131	172
	Africa	135	176	146	180
	North Africa	160	216	149	196
	Latin America	134	177	140	185
Developing Regions		134	170	152	192
Developed USSR		14	14	14	14
North America		14	14	14	14
Oceania		14	14	14	14
World		152	198	180	220

Although efforts may be made in various ways to meet and close the protein gap, the only solution to be discussed here is increasing available supplies of protein foods, particularly cereals. Cereals constitute staple food of 90% population in the developing countries. Here are the percentage contributions of cereals to protein supplies in the principal developing countries of the World.

Brazil - 5%	Africa - 5%	South America - 7%
Near East - 6%	Europe, Pakistan - 6%	Thailand - 5%
Eastern Europe - 5%	Mexico, Central America - 4-4.5%	Far East World - 4%

Hence the principal method of increasing production of crude protein is to promote the production of cereals. Although the protein content of most of the cereals exceeds 10%, their NPU is only between 50 and 55%. It is therefore necessary to improve the quality of the diet by simultaneous production of certain quantity of protein foods other than cereals (pulses, animal products) for supplementation.

It is necessary to pay particular attention to the protein quality of selected seeds. A variety of maize known as Opaque 2 (highly in high lysine variety) has already been produced. There are limiting amino acids in these cereals too. Analogous results have been obtained both in Philippines and in Guyana by producing IR 8 variety of rice. The protein content of the rice (IR 8) produced by Dr. Pawar in Guyana was 11.12%, these were high yielding short

variety. Mexico was poorest in producing high yielding wheat. The results of supplementation are shown in Table 6.

TABLE 6

Influence of protein content of feed on nitrogen requirement (g per cup of feed per day)						
Country (Dietary composition)	Total protein of diet g/day	Protein % of diet	Source of protein	NPU <sup>a</sup>	Nitrogen requirement g/day	Nitrogen intake g/day
<b>IRAN</b>						
(1) Common wheat (Pr 11.4%)	72.4	11.4	Common	5	5.8	19.8
(2) Wheat (Pr 11.4%)	81.7	11.4	Common	5	6.3	42.6
(3) Family diet (11+10% of (2))	89.4	11.4	Family diet	51	6.3	46.1
<b>PHILIPPINES</b>						
(1) Local rice (Pr 7.3%)	42.3	11.1	Sulphur	59	6.3	28.5
(2) HYV (Pr 11.4%)	51.7	11.4	HYV	51	7.6	28.7
(3) Family diet (11+10% of (2))	56.9	11.4	Family diet	55	6	31.1
<b>GUATEMALA</b>						
(1) Common maize (Pr 9.5%)	72.0	12.6	Common	49	8.1	33.3
(2) High yielding maize (Pr 11.4%)	78.8	11.4	High yielding	51	8.1	46.3
(3) Family diet (11+10% of (2))	86.7	11.4	Family diet	59	8.1	49.4

It is possible to deduce from the results shown in this Table 6 that

1. Higher protein content does not necessarily mean higher NPU of the diet (see Iran). The maximum protein content of a protein source and in no way proportional to higher protein intake because of the poor quality of the protein in the cereals.

2. A maximum protein intake level is obtainable without necessarily any increase in yield mainly through the improvement of protein quality (high yielding variety maize in Guatemala).

### FINAL COMMENT

It is well known that the targets and objectives are rarely attained. For India even with most optimistic prospect for the rise of individual income, protein consumption in 1975 will rise by one-third and in 1975 only by two-thirds of the increase envisaged in per capita requirement (P). According to the same calculations another 25 years of sustained economic development would be necessary to attain a production level such that a great majority of the people will have an adequate protein intake. The Indicative World Plan of Agriculture and Development reaches a pessimistic conclusion for most part of the world.



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## NEUROCHEMISTRY OF MALNUTRITION\*

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### *Prenatal malnutrition produces lifelong mental blight*

The infantile malnourished population is increasing because it covers the period during which malnutrition produces both damaging effects that may persist and be passed down to later years. Battered children are out of their mothers' arms, they are mostly victims of malnutrition in India, Pakistan, Bangladesh, Biafra, China, Mexico, Puerto Rico and elsewhere throughout the world. Those who manage to survive may be found to suffer mental blight. Numerous experimental and clinical findings have pointed sharply towards the conclusion that malnutrition imposed during critical period of development produces permanent changes in brain structure and function. Neurological abnormalities such as apathy and lethargy are prominent symptoms of severe malnutrition in children. Biochemistry and noninvasive electroencephalography have been extensively reviewed by Womels and Chomby.<sup>1</sup> Some important neurochemical changes that occur in malnourished children are discussed in this review. Some serious changes have already produced high risks of retardation of mental development as a result of persistent malnourishment. These changes have enormous implications for about 460 million children living in food-deficient underdeveloped areas of the world and growing on or near calorie diets. These are all the more disturbing as far as the future of malnourished children of India is concerned.

### *Prenatal malnutrition decreases brain's cellular contents*

The number of brain cells becomes final at birth. It is not therefore unlikely that dietary restriction, particularly that of protein, during gestation of mother, may result in some permanent reduction in brain cells. Zimhoffer and associates<sup>2</sup> at Los Angeles University of California School of Medicine checked this point by carrying out an animal experiment with female rats maintained on 8% or 27% protein diet by postnatal schedule for one month prior to mating and through entire gestation period. The investigators observed that the brains of newborn rats from the females on the 8% protein diet contained significantly less DNA and protein compared to the progeny of the females on the 27% protein diet. Further, there were fewer brain cells, and

the protein content per cell was lower in newborns of protein-deprived mothers. In a more recent experiment, Low and Oliva fed either 6%, or 18% protein diet to timed pregnant rats, and at 14 days of pregnancy. The pups were sacrificed on day 2, 4, 5, and 21 respectively. At all stages cerebral contents of RNA, DNA, and protein were lower in the malnourished pups than pups suckled by adequately fed dams. In the same RNA, DNA, and total protein were used 36, 14, and 20.6% respectively between the days 2 and 21 compared with increases of 45, 70, and 80% in the adequately fed pups. For the cerebellum, the increases in aspartic acid, glutamic acid, and serine and leucine were also lower in the malnourished than in the well-fed pups. For the protein content of the cerebellum, the malnourished pups showed a 1-hour delay in which it was less than in the well-fed pups than a well-fed pup. Nutrition during the period of rapid postnatal brain growth may determine brain cell content. Howell and Granoff subjected mice to nutritional restriction by removing them from their mothers during the period of rapid postnatal brain growth (2 to 12 days of age) and replacing them with a 5% protein diet. A 50% reduction in body weight was produced by day 12. Thereafter they were fed a 18% diet. At the end of the study, cerebellum and cerebral weights were reduced by 17% and 4% respectively compared with the normal controls. Total DNA was reduced 30% in the cerebellum and 15% in the cerebrum. Thus, even mild malnutrition at the time of rapid brain growth brings about a lasting decrease in brain size, in the number of brain cells, and their cellular contents. Nutrition improvement afterwards does little or nothing to correct the damage that has already occurred as a result of malnutrition at a critical period of development. In contrast, some systems recover after the critical period has passed, thus the damage is partially reversible. There are limits of brain growth which may restrict the corrective chronological time and if this opportunity is missed due to nutritional or other restrictions, complete rehabilitation may not be possible. In rats and mice, the period of fastest brain growth is believed to be the first few weeks after birth. In man, the corresponding period of rapid brain growth is believed to be the last few weeks before birth. Clinical data point out that undernutrition in early human life results in the reduced brain size and weight.

#### ***Malnutrition lowers brain DNA***

Because DNA content of cells is constant for each species, it is possible to determine the number of cells in a tissue sample by measuring the DNA content. By plotting normal DNA values, Dr. M. Wozniak of New York's Cornell University Medical Center demonstrated that cell density in human brain slowed at birth, but continued until about 6 months. Thus, rapid







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Khan of Dacca (another modern doctor interested in the study of ancient Arabic and Urdu medicine) found the alkaloid in the gum, white and black, prepared with a mixture of the drugs. He was the first to isolate the virtues of this remedy and he induced two modern chemists, Siddiqui and Siddiqui (1936-37), to undertake a systematic study of the chemical composition of the drug. Another chemist, a disciple of Sen and Bose, R. N. Chatterjee at the Calcutta School of Tropical Medicine started a series of chemical and pharmacological investigations in cooperation with Siddiqui and myself. The two chemists isolated 5 crystalline alkaloids, 2 from the gum and 3 from the bark. A qualitative analysis of the alkaloids was carried out by the Calcutta group produced a total alkaloid of 1.5% and also an alkali of a mixture of alkaloids with a content of 1.5%. The above progress supported the belief in the drug is with the material found for a comprehensive pharmacological investigation.

#### *Early Pharmacological Investigations \**

After more than one year of delay, the first paper was published by Chatterjee, Gupta and Mukherjee in 1937 in the *Indian Journal of Medical Research*. The conclusions arrived at from the preliminary pharmacological evaluation made in the laboratory were as follows:

- "The alkaloid *guttal* R. S. exerts a marked effect on the plain muscle of the intestine and the uterus."
- "The systemically blood pressure falls due to depression of the blood vessel of the sympathetic nerve. The respiratory depression is due to the effect from failure of respiration due to the paralytic effect on the respiratory centre."
- "The alkaloid has a pronounced effect on the central nervous system. In sublethal doses injected into the peripheral of the brain, various effects are observed. In mammals the alkaloid produces symptoms which are attributable to a depressing effect on various cerebral centres in the reverse order of their development. The first period of excitement seen in guinea-pigs and cats is probably due to the depression of the higher centres as well as to a direct action on the brain and a central. There is also evidence to show that there is a depression of a nerve cells in the body."
- "The alkaloid on account of its cerebral depressant properties should prove to be a valuable sedative drug. Its depressant effect on the respiratory centre should, however, be borne in mind. It lowers the blood pressure and if administered in proper dosage should be of value

remedy is to be found in the fact that the nation is not faced by a single problem, but by a series of problems. The first of these is the problem of the national debt. The second is the problem of the national income. The third is the problem of the national expenditure. The fourth is the problem of the national balance of payments. The fifth is the problem of the national foreign trade. The sixth is the problem of the national foreign investment. The seventh is the problem of the national foreign exchange. The eighth is the problem of the national foreign reserves. The ninth is the problem of the national foreign assets. The tenth is the problem of the national foreign liabilities. The eleventh is the problem of the national foreign equity. The twelfth is the problem of the national foreign debt. The thirteenth is the problem of the national foreign interest. The fourteenth is the problem of the national foreign dividend. The fifteenth is the problem of the national foreign profit. The sixteenth is the problem of the national foreign loss. The seventeenth is the problem of the national foreign gain. The eighteenth is the problem of the national foreign surplus. The nineteenth is the problem of the national foreign deficit. The twentieth is the problem of the national foreign balance.

The first of these problems is the problem of the national debt. The second is the problem of the national income. The third is the problem of the national expenditure. The fourth is the problem of the national balance of payments. The fifth is the problem of the national foreign trade. The sixth is the problem of the national foreign investment. The seventh is the problem of the national foreign exchange. The eighth is the problem of the national foreign reserves. The ninth is the problem of the national foreign assets. The tenth is the problem of the national foreign liabilities. The eleventh is the problem of the national foreign equity. The twelfth is the problem of the national foreign debt. The thirteenth is the problem of the national foreign interest. The fourteenth is the problem of the national foreign dividend. The fifteenth is the problem of the national foreign profit. The sixteenth is the problem of the national foreign loss. The seventeenth is the problem of the national foreign gain. The eighteenth is the problem of the national foreign surplus. The nineteenth is the problem of the national foreign deficit. The twentieth is the problem of the national foreign balance.

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In 1910, Chapin, Gurn, Ross, and Chase, received their training in the hypnosis principles of tar paper. As a result of this study they revised the earlier conclusion arrived at by Chapin and Chase, regarding the sedative properties of serpentine. This decided was found to be a CNS stimulant similar to muscimine and sepiamine. Regarding the nature of the hypnosis and

sedative principle of the plant was suggested that the most valuable fraction of fractions other than the alkaloid extract was the whole plant extract for this property of the plant.

In 1944, Gupta, Kishore and Datta examined the whole plant extract due to the hypnotic principle of *Rauwolfia serpentina*. They also recorded that the oleoresin fraction from plant extract was found to be more effective at that time produced sedative and hypnotic effects in cats, rats, dogs and guinea-pigs. The effect of the extract was also compared by the standard extract commonly used and administered in a dose of 2 mg. per lb. in 24 hrs. These workers also mentioned the effect of drawing 'reserpine' from the new plant that was later isolated and named by the Swiss workers working on the plant as 'reserpine' the most important alkaloid in *Rauwolfia*.

### Early Clinical Observations :

Professor Chandra was a physician with a long experience in the treatment of mental and as soon as the crude extract of the plant was prepared in the laboratory and a few preliminary experiments were made, attention could be worked out by him and his assistants that the crude standardized crude extract of the whole plant extract might be useful. On the patients under his care at the Government Hospital for Mental Diseases, Chandra and Gupta noticed a variety of effects of the drug obtained in the Pharmacology Laboratory and he was particularly struck by the prolonged sedative effect of the drug. But he and Maheshwari and Jettok collaborated with the Director of the Royal Medical College in Bonn a controlled clinical trial of the drug in severe cases of violent disorders showing violent manic symptoms. Results obtained in the cases of schizophrenia were encouraging but uniform results did not withstand the test of critical analysis were not available. In one set of trial actually a recrudescence of violent symptoms with increased agitation and excitement was recorded. In view of the sedative nature of the drug Chandra discouraged the publication of the drug as needed by the hospital at that time. Memories connected with the *Rauwolfia* drug were confined outside the Hospital and through his influence Chandra was able to get reports of these cases noted by private physicians in the general hospital clinics. An overall analysis of the first six years from these sources led little room for doubt that *Rauwolfia* had a definite contribution to therapy particularly in cases with high blood pressure and manic depression symptoms.

Since the reported efficacy of the whole plant extract of *Rauwolfia* on an anxiety by Sen and Bose, many workers started with the plant the drug



vide Rawson's work. A further study had been made previously by Lederer and his group. It was to improve the new compound by further substitution. In this we started the whole process over again. First, the absolute configuration was determined by x-ray crystallography at 20° C. and the compound was found to be the absolute enantiomer. We started the synthesis again.

The product was crystallized from a mixture of petroleum ether and benzene. The yield was 100 mg. (10%). The melting point was 100° C. The compound was found to be the absolute enantiomer. A further study of the absolute configuration was made by x-ray crystallography. The results have been given."

## (2) *Preparation of the compound from the starting material*

The compound was prepared by the reaction of the starting material with the reagent. The reaction was carried out in a round-bottomed flask equipped with a magnetic stirrer and a reflux condenser. The reaction mixture was stirred for 24 hours at room temperature. The reaction mixture was then poured into a large volume of water and extracted with ether. The ether extract was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The pure compound was obtained as a white solid.

The compound was then subjected to a series of reactions. The compound was first treated with a solution of sodium hydroxide in ethanol. The reaction mixture was stirred for 24 hours at room temperature. The reaction mixture was then poured into a large volume of water and extracted with ether. The ether extract was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The pure compound was obtained as a white solid. The compound was then subjected to a series of reactions. The compound was first treated with a solution of sodium hydroxide in ethanol. The reaction mixture was stirred for 24 hours at room temperature. The reaction mixture was then poured into a large volume of water and extracted with ether. The ether extract was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The pure compound was obtained as a white solid.

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These observations are to be taken into account in the future. The compound was then subjected to a series of reactions. The compound was first treated with a solution of sodium hydroxide in ethanol. The reaction mixture was stirred for 24 hours at room temperature. The reaction mixture was then poured into a large volume of water and extracted with ether. The ether extract was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The pure compound was obtained as a white solid. The compound was then subjected to a series of reactions. The compound was first treated with a solution of sodium hydroxide in ethanol. The reaction mixture was stirred for 24 hours at room temperature. The reaction mixture was then poured into a large volume of water and extracted with ether. The ether extract was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. The pure compound was obtained as a white solid.







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## 5. (b) IN, FIVE, SIX, AND TEN, USING SYSTEM

O C CHAIRMAN

Calcutta University

from Late Professor B. C. Gupta

## VITAMIN C METABOLISM IN ANIMALS

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Research on various pathologies related to the endocrine and metabolism of the hypothalamus have been carried out and a new hypothesis on the definition

The above results suggest that the use of a single word  
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Springer-Verlag, Berlin Heidelberg New York, 1983.

In my opinion, however, a more effective way to deal with the problem of child labor would be to increase the minimum wage for children. The problem of child labor is caused by the fact that many parents are poor and need the help of their children to survive. On the other hand, the government has a duty to protect the children from exploitation.

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### CONDITIONS

Research on the role of the nonverbal communication system in the process of social interaction has been limited. The present study was designed to investigate the role of the nonverbal communication system in the process of social interaction. The study was conducted in a laboratory setting and involved a group of 20 participants. The participants were asked to interact with a computer program that simulated a social interaction. The program was designed to provide feedback on the participant's nonverbal communication behavior. The results of the study showed that the nonverbal communication system played a significant role in the process of social interaction. The participants who received feedback on their nonverbal communication behavior showed improved social interaction skills compared to the control group. The findings of this study have important implications for the development of social skills training programs.

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PRITTSVILLE, N.Y. 13821

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1. The first step in the process of creating a new product is to identify a market need. This involves conducting market research to understand the preferences and behaviors of potential customers. Once a need is identified, the next step is to develop a concept that addresses this need. This concept should be unique and offer a clear value proposition to the target market.

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reported that a small amount of poly(A) could be incorporated into a mixture of ribosomes and amino acids, but that the incorporation of C-<sup>14</sup>-poly(A) was very low (12). The incorporation was further increased by the addition of a small amount of poly(U) or poly(C) to the mixture, but this was considered as the permeation of poly(A) mRNA into the ribosomes rather than as a permeable. In the case of poly(A) incorporation, the incorporation of poly(A) required a small amount of poly(U) or poly(C) RNA.

### *Effect of different inhibitors*

The effect of various inhibitors on the incorporation of a small amount of poly(A) into a mixture of ribosomes and amino acids was studied. The results are shown in Table I. The incorporation of poly(A) was inhibited by the addition of a small amount of poly(U) or poly(C) RNA, but not by the addition of a small amount of poly(A) RNA. The incorporation of poly(A) was also inhibited by the addition of a small amount of poly(U) or poly(C) RNA, but not by the addition of a small amount of poly(A) RNA.

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### *Effect of different concentrations*

The effect of various concentrations of poly(A) on the incorporation of a small amount of poly(A) into a mixture of ribosomes and amino acids was studied. The results are shown in Table I. The incorporation of poly(A) was inhibited by the addition of a small amount of poly(U) or poly(C) RNA, but not by the addition of a small amount of poly(A) RNA. The incorporation of poly(A) was also inhibited by the addition of a small amount of poly(U) or poly(C) RNA, but not by the addition of a small amount of poly(A) RNA.

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## Membrane Bound Enzymes of Brain

A second method of determining the relative contribution of the different components of the total variance to the total variance is to calculate the relative contribution of each component to the total variance. This is done by dividing the variance of each component by the total variance. The results are shown in Table 1.

From the above data it is evident that the  $\alpha$ -ketoglutarate concentration in the plasma is not significantly different from the normal range. The plasma concentrations of lactate, pyruvate, and ketone bodies at the different stages of uremia are similar to the values reported by other workers in the field of renal biochemistry.





The gold glycolated on acid heifer virus yielded glucosamine, galactose, glucose and ceramide. The gold and virus R value from that of the Fersman hapten on thin layer chromatography.

The terminal group of gold glycolated was suspected to be D-glucose as it inhibited agglutination between blood and anti-B serum. It is confirmed that D-glucose is the terminal group of gold glycolated as it was treated with the ceramide D-glucose hydrolase and the released glucose was estimated. The second terminal group was identified to be N-acetyl D-glucosamine which was released by ceramide D-glucose hydrolase treatment was found to be a terminal group of virus glycolated. N-acetyl D-glucosamine hydrolase of gold glycolated. The results of the above successive treatments of the above mentioned compounds showed that virus D-glucose and D-glucose-ceramide as its R value is very close to virus glycolated on thin layer chromatography. The ceramide D-glucose hydrolase glycolated a toxin D-glucose (1:4) O-N-acetyl D-glucose (1:4) and D-glucose (1:4) C-2 D-glucose-ceramide and a delayed form of virus glycolated on Fersman hapten.

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# PRODUCTION OF PROTEIN BY SUBMERGED GROWTH OF EDIBLE MUSHROOMS

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## Introduction

India and elsewhere resort to various means to make use of many "non-conventional" sources of food, such as insects, earthworms, snake and fish meal products, leaves and grasses and a number of other non-traditional food systems. Out of these mushrooms are the most popular, but not yet fully realized potential source of food. They are rich in protein and vitamin. The suitability of mushrooms as a food material is based on three attributes: (a) safety, (b) possibility of a large scale multiplication and growth by microorganisms and (c) the fact that they are easy to cultivate. (a) Good record of production of mushrooms and their nutritive properties of the fermentation technology which has been developed as a simple and cheap method using not twenty very numerous of microorganisms.

Microorganisms which may be attracted to mushrooms are fungi, bacteria (from water and mineral salts), bacteria and fungi. Some of these are already being consumed by insects, the rest of them produce protein.

## Mushrooms as food

Edible fungi of human consumption are the most common and are food. The fleshy fruiting bodies of mushrooms are rich in protein, vitamins and minerals as well as high protein value and are not harmful. Some of the most popular edibles are *Agaricus bisporus*, *Agaricus campestris*, *Agaricus bisporus*, the shiitake mushroom *Lentinula edodes*, the oyster mushroom *Pleurotus ostreatus*, the king of the forest *Cantharellus cibarius*, the honey mushroom *Armillaria mellea*, the parasol mushroom *Lepista nuda*, the honey mushroom *Armillaria mellea*, the vesicle stemmed mushroom *Cantharellus cibarius*, the morel *Morchella esculenta*, *Cantharellus* and porcini. Most of these mushrooms grow in wild and collected from their natural habitat. Planted cultivation of some selected popular species are practised to get a definite quantity of mushroom fruit bodies of uniform quality at large scale.



to be in, but the more exact definition of an influence free environment is a natural one. The influence produced by an external world is not the same as the influence of a human agent. A human agent is a complex of many influences and traces of a comparison are made between the influence of the external world and the influence of the human agent, and the result is a comparison of the influence of the external world and the influence of the human agent.

The equilibrium constants of the reactions (1) and (2) were tested in the same way as the equilibrium constants of  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Ni}^{2+}$  by means of the comparative method. As a result, the equilibrium constants of reactions (1) and (2) were determined to be  $1.0 \times 10^{-10}$  and  $1.0 \times 10^{-11}$ , respectively. The equilibrium constants of reactions (3) and (4) were determined to be  $1.0 \times 10^{-10}$  and  $1.0 \times 10^{-11}$ , respectively. The equilibrium constants of reactions (5) and (6) were determined to be  $1.0 \times 10^{-10}$  and  $1.0 \times 10^{-11}$ , respectively. The equilibrium constants of reactions (7) and (8) were determined to be  $1.0 \times 10^{-10}$  and  $1.0 \times 10^{-11}$ , respectively. The equilibrium constants of reactions (9) and (10) were determined to be  $1.0 \times 10^{-10}$  and  $1.0 \times 10^{-11}$ , respectively. The equilibrium constants of reactions (11) and (12) were determined to be  $1.0 \times 10^{-10}$  and  $1.0 \times 10^{-11}$ , respectively.

$\frac{1}{2} \pi$

The growth of *Ch. impatiens* S. & W. in 1961 was very similar to the slow growth of the control in 1960. The maximum value of growth of the control in 1960 was reached by the end of the period of observation and could be due to a high degree of photosynthesis on the rate of absorption of water and nutrients from the soil and rate of chlorophyll of the medium. The control was harvested and the growth of the control was very low. The control could be harvested after 144 hours and the growth of the control was very low.

117. 1. I found green L. hirsuta on it, in fruit.

[illegible]

of a fermentation of these media by which fermenters in the culture media for the isolation of *Aspergillus fumigatus* S. 1001b, very small fungi A K and A B B. *Aspergillus fumigatus* S. 1001b is the predominant fungus.

*Nutritive value of medium and medium fraction obtained from submerged fermentation*

The medium of 4 *Aspergillus* S. 1001b from submerged culture is a rich source of riboflavin, niacin, and vitamin B<sub>12</sub> and is rich in iron and calcium content. Amino acid content of medium is dependent on culture age and reaches maximum in 7th day of fermentation. The free content of dried medium varied between 42.1 to 45.5 per cent. Medium from older culture have higher free amino acid. Protein content from the medium is 10.5 per cent. All amino acids except glutamic acid, aspartic acid, and proline are in low concentration but have a high percentage of valine, leucine, and isoleucine. Biological score and nitrogenous and fat for the purified protein are 75, 37.8 and 58.4 respectively (13).

### Conclusion

It is a well known fact that the people of the low income groups in developing countries like India do not receive adequate amounts of protein of high quality and that consequently protein deficiency is widely prevalent. The problem of utilization of available additional sources of proteins for vegetarian consuming Indians in the face of Indian people has engaged the attention of food scientists of the country during the past quarter century. The present study was undertaken to develop a suitable strain of 4 *Aspergillus* for submerged culture. The fermentation conditions of the strain so developed for maximum protein production were standardized.

The selection of the maximum 4 *Aspergillus* isolated mainly on two reasons: (a) A good amount of riboflavin, niacin, and vitamin B<sub>12</sub> can be submerged cultivation of this organism. The growth characteristics were characterized by Hamfeld and Shrivastava (14). The presence of this fungi in Indian soil in India no attempt is made to isolate maximum of this organism. (b) A source of protein for vegetarian consuming Indians. It will be to test and nutritive value of this fungus.

The most important factor determining the biological value of a protein is the relative concentration of essential amino acids. The essential ones present in it. Protein of 4 *Aspergillus* S. 1001b is a rich source and contains the concentrations of these amino acids exceed that of reference protein recommended by FAO. The essential amino acid made up of 4 *Aspergillus* S. 1001b protein compares favourably with those of protein from different other micro-

original. I have not seen any other specimens of this species and judge it to be that of *S. cereale*.

[illegible]

### Acknowledgements

The works in this category have received much attention and have been widely used with a few exceptions.

## References

- [illegible]



[illegible][illegible]

(b) Thiaminase II :

It is present mainly in the bacterial system such as *Bacillus cereus*, *B. subtilis*, *Ustilago*, *Uromyces*, *Aspergillus*, *Penicillium*, *Trichoderma*, etc. (15). The enzyme II has been successfully isolated from *Bacillus cereus* and is more like that of a whole molecule (16). It is an enzyme of a relatively low molecular weight crystallized by Ishihara (16). The amino acid composition (section 47) indicates a simple protein with the absorption maximum at 276 and a minimum at 252. Various physicochemical properties of the enzyme determined to its molecular weight of 164,000 (16). *Bacillus* is a thermophilic organism to spot the enzyme into two isoenzymes without the participation of cofactor (18). The enzyme activity is measured (19) after incubation in a phosphate buffer and enzyme followed by the determination of residual substrate by titrimetric method.



ortho to para position did not change significantly whereas with the change of a free to a fixed position, the change in  $\rho$  was not statistically significant and was negligible.

[illegible]

Various authors have proposed that the structure of the side chain of "Frustrated" is a branched alkyl chain, with a terminal methyl group. This is based on the observation that the side chain of "Frustrated" is branched, and the terminal methyl group is the only one that is not part of the main chain. The structure of the side chain of "Frustrated" is shown in Figure 1. The side chain of "Frustrated" is a branched alkyl chain, with a terminal methyl group. This is based on the observation that the side chain of "Frustrated" is branched, and the terminal methyl group is the only one that is not part of the main chain. The structure of the side chain of "Frustrated" is shown in Figure 1. The side chain of "Frustrated" is a branched alkyl chain, with a terminal methyl group. This is based on the observation that the side chain of "Frustrated" is branched, and the terminal methyl group is the only one that is not part of the main chain. The structure of the side chain of "Frustrated" is shown in Figure 1.

More than 200 people (100 men, 50 women and 50 children) were involved in the project. The project was a success and the children were able to learn the value of hard work and the importance of the environment. The project was a success and the children were able to learn the value of hard work and the importance of the environment.

[illegible]

The infrared analysis and mass spectrum shows the molecular formula of  $C_{10}H_{10}O_4$ , having no nitrogen in its composition. The infrared analysis indicates the presence of a carbonyl group at  $1700\text{ cm}^{-1}$  and a conjugated carbonyl at  $1600\text{ cm}^{-1}$  and a hydroxyl group at  $3400\text{ cm}^{-1}$ .

The identity of the compound was confirmed by the NMR spectrum. A methyl group was indicated by a singlet and a conjugated trans double bond (AB quartet) at  $\tau 6.9$  and  $\tau 6.1$  and a conjugated aromatic proton at  $\tau 7.2$  and  $\tau 7.4$ . The presence of a methoxy group (6 protons) was indicated by a singlet at  $\tau 3.8$ . Analysis shows that the compound is 3-methoxy-4-hydroxy-2-pyridone-5-carboxylic acid.

The compound was prepared by the reaction of 3-methoxy-2-pyridone with acetic anhydride. The reaction was carried out in a round-bottom flask as well as in a test tube. The reaction was carried out in a round-bottom flask to study the reaction of the compound with acetic anhydride and to determine the pyridyl group under various conditions. The compound was isolated by heating the reaction mixture in a round-bottom flask with a magnetic stirrer and an overhead stirrer. The compound was isolated by heating the reaction mixture in a round-bottom flask with a magnetic stirrer and an overhead stirrer.

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When is the fish assemblage more diverse than in the past? from the same habitats to the present? The answer is that it is not. In fact, the same 36 fish species have been found in the same habitats in the early 1960s. The present diversity is somehow linked with the vegetation, the stream morphology and the environment. The estimation of the loss of biodiversity required loss of species is now calculated on the up. The estimation of minimum richness to the greatest complexity. This is calculated with the realized capacity  $\hat{S}$  with  $z = 1.5$  the

When the descendants of *Crinoid* (a *Crinoid*) first came out of the water and crawled upon the mud banks, they were not at all fitted for Devonian periods to become the dominant life forms. They were not fitted for a completely new course of evolutionary development. They were not fitted for the aquatic to the terrestrial mode of life with a prolonged, almost boundless, enormous range of adaptations under strong selection pressure. The primitive quadruped vertebrates had to face a variety of extreme structural changes like supporting of body weight, locomotion on a land, posture, a long expansion, desiccation by dry air and hot sun. Perhaps the re-uptake of sea-



INCAPABLE  
LIVER  
KIDNEY  
INCAPABLE

PRIMATES

MAMMALS

BIRDS  
(HIGHER ORDER)

BIRDS  
(LOWER ORDER)

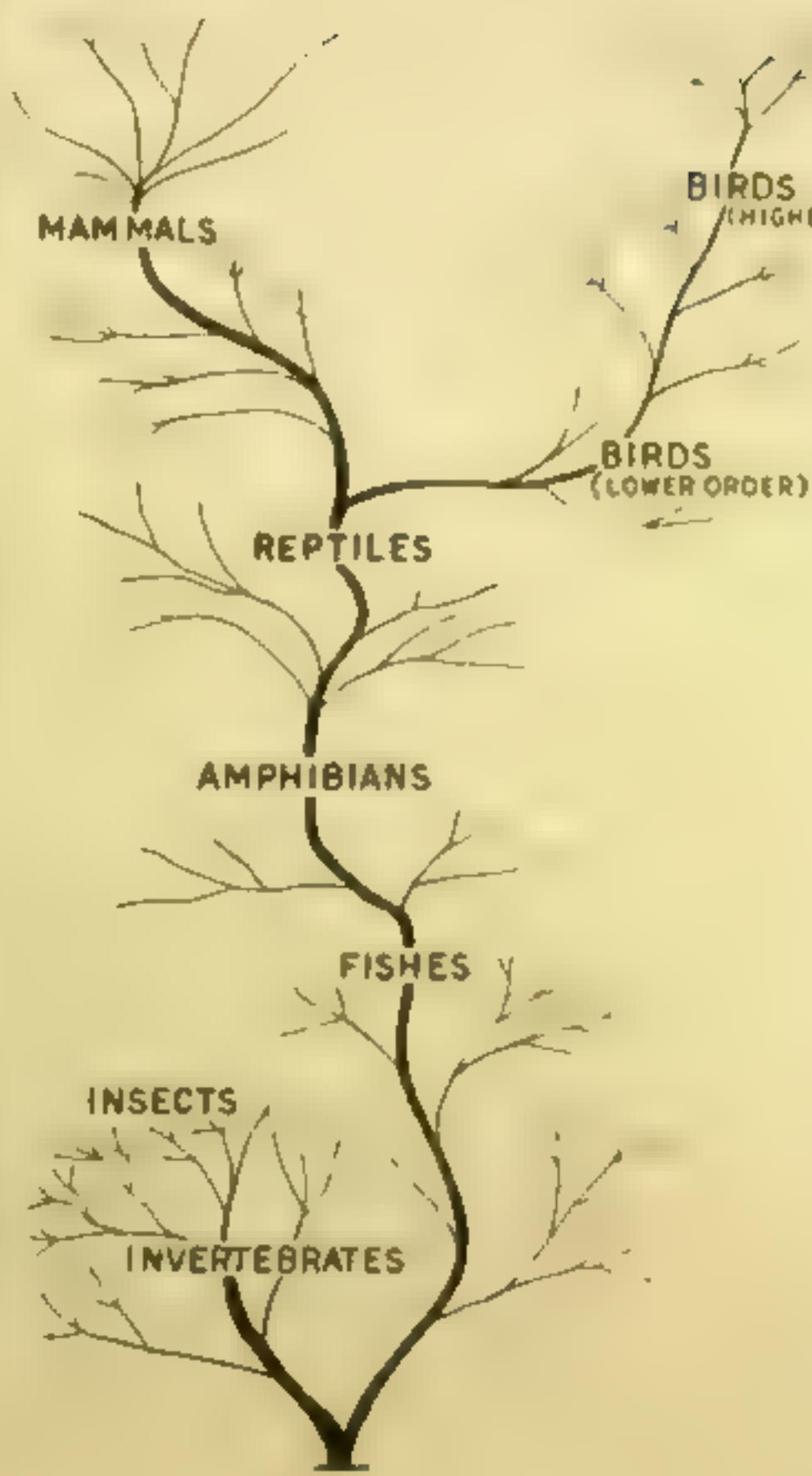
REPTILES

AMPHIBIANS

FISHES

INSECTS

INVERTEBRATES



## NEED FOR ASCORBIC ACID

The dietary intake of ascorbic acid for maintenance of health is a subject of considerable controversy. The average person consumes it all present in the diet, about 100 mg a day in man's diet, usually 50 mg a day. However, studies of the ascorbic acid content of plasma have shown that the average concentration is 0.1 mg per 100 ml. Food and Nutrition Board of the National Research Council (18) has estimated that the daily intake of 75 mg may be sufficient for the maintenance of plasma level.

In contrast to the above, Pauling (22) has estimated that the minimum concentration of ascorbic acid in the blood should be 2.0 mg per 100 ml. He estimated that the minimum concentration of ascorbic acid in the blood should be 2.0 mg per 100 ml. According to Pauling, the fact that the minimum concentration of ascorbic acid would indicate that for optimum health, the need for ascorbic acid is much more than that which can be provided by the average person's diet. Pauling considered that there is a margin by which the concentration of ascorbic acid in the blood is very low, so that if the concentration of ascorbic acid in the blood is low, the rate of ascorbic acid production is low. He estimated that the rate of ascorbic acid production in man is 28-56 mg per kg body weight per day (22, 24). A person with a body weight of 70 kg would produce 1.96-3.92 g of ascorbic acid per day. Pauling (22) estimated that a person weighing 70 kg should have a daily intake of 1.8 g of ascorbic acid per day. Pauling (22) further assumed that the average concentration of ascorbic acid in the blood is 0.1 mg per 100 ml. He estimated that the average concentration of ascorbic acid in the blood is 0.1 mg per 100 ml. To support this, he cited the example of Bourne (25) that the gorilla consumes about 4.5 g ascorbic acid per day.

However, the arguments of Pauling are questionable. The results presented elsewhere (14) would indicate that the biosynthetic capacity of mammals decreased with progress of evolution. Since the biosynthetic capacity would apparently depend on the need, it may be well assumed that the need for ascorbic acid has decreased with the progress of evolution. Actually, studies with labeled ascorbic acid has shown that whereas the daily requirement for ascorbic acid per kg body weight of rats is 26 mg, that of the guinea pig is 9 mg and of human being only 1 mg (26). In his article on "Evolution and the need for ascorbic acid," Pauling (22) did not consider the possibility that the need might decrease with the progress of evolution. There was little relevance to compare the need of man with that of the rat which is about 26 times more



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Planning for an adequate nutritional status through dietary means for a nation will certainly include the fact that improvement of the national food is one of the main tasks of agriculture. It is a task which is concerned by many people except the agriculturists themselves. The government, the industry, the commerce, the education, the medicine and the family will all try to reach to the agricultural development and attended with the agricultural development. The government will try to pay attention of food production and distribution, the industry will try to take foods which are necessary for the production of the goods, the commerce will try to make a market for the foods, the education will try to make an amount of nutritional knowledge, the medicine will try to make a knowledge of the advantage in diet. These are the main tasks of the agricultural cost. These brief arguments will be enough to show the importance of the improvement of foods.

Roy advocated that we improve our food supply. The need is still there for food products, especially in the Pacific because of increased consumption by the military. It is possible to increase it. We can do this by increasing production. It can be done through better management and more efficient methods. It should always be regarded as a war effort. A war effort can be considered for national security.

## CEREALS, PULSES AND THEIR PRODUCTS

In India at present enriched flour and rice are known and used. The specifications along with others have been laid down in Food Rules, 1955 (6). Two other products, malted sorghum and malted rice, have been tailored with an eye to the special needs of the population. These products have not been laid down in the Food Rules, 1955. The flour is enriched with lysine, niacin and vitamin B1 but it is not a complete dietary product.

But these existing activities can be extended by introducing new foods which may include white rice and wheat breakfast cereals and nutritional products. Whole rice and wheat can be enriched with essential vitamins, riboflavin and niacin (with a coating of emulsion for rice) - premix or mastermix to be blended with rice and atta respectively at various mills or by the processors. Riboflavin may be extended to extend in rice as it was found to be expected in Iloilo and Manila that rice with yellow spots were not liked by the housewives at the beginning who used to pick them out and discard them. However later it was reported that not only had the consumers accepted the yellow spots been removed but that some said the yellow colour gave a sense of security (").







## S. K. Baid

Department of Geo. &amp; A. S. 1881-1882

Since the discovery of penicillin, the treatment of bacterial diseases has been greatly improved. However, the work on mycobacteria was not so successful. The results were already reported before the formation of the International Union of Pure and Applied Chemistry. Mycobacteria were found to be resistant to penicillin.

[illegible]

The antibiotic is a cyclic polypeptide consisting of 13 amino acids linked by amide bonds. Its diffusion rate through a membrane was determined by the method of Klotzel (1958) and was found to be 1.5 × 10<sup>-6</sup> cm<sup>2</sup>/sec when calculated from the molecular weight of 1300. The molecular weight of the antibiotic was determined by the method of Klotzel (1958) and was found to be 1300. The molecule which is secreted by the bacteria is a cyclic polypeptide consisting of 13 amino acids linked by amide bonds. The amino acids were sequenced by the method of Klotzel (1958) and were found to be: L-alanine, L-proline, L-phenylalanine, L-tyrosine, L-tryptophan, L-isoleucine, L-valine, L-leucine, L-methionine, L-glutamine, L-glutamic acid, L-aspartic acid, and L-asparagine.

Mycobactin was purified by column chromatography on a two-dimensional paper chromatography system using a complex mixture of peptides. A mixture of 15 peptides, including six tripeptides and two tetrapeptides, was used. The N-terminal residue of each peptide was determined by the phenyl isothiocyanate (DNBP) method. The amino acid sequence of the peptides without the peptide backbone was determined by the method for determining C-terminal amino acids by the use of the amino acids such as aspartic acid, glutamic acid, and the proportion of one molecule each of the amino acids. Amino acids occur only once in the sequence of the known peptides.

rides containing any one of these amino acids has been helpful in determining the possible arrangement of amino acids in tripeptides and tetrapeptides. This knowledge of chemical structure of di-, tri- and tetra-peptides has made it possible to fit together 15 peptide fragments from 13 amino acid residues of mycobactin into a linear sequence (Fig. 1). In the absence of free L amino group in the molecule, a cyclic structure has been suggested<sup>1</sup>.

At the present stage of knowledge of the molecule and 4 out of the 5 residues of aspartic acid are D. Determination of the D/L sequence of L-amino acids. Thus the D/L sequence of amino acids in mycobactin resolves itself to the determination of the D/L sequence of aspartic acid only. The treatment of a few tentatives of aspartic acid containing peptides, obtained from partially hydrolysed mycobactin with D-amino acid oxidase or L-plataminc acid decarboxylase (containing L-aspartic decarboxylase activity) revealed L-aspartic acid only in all peptides and D-aspartic acid in others. Hydrazinolysis show that one such L-aspartic acid containing peptide is aspartyl-tyrosine and the other is Asp-tyr-tyr. Majumdar and Bose had shown by reaction with diphenyl carbodiimide that the peptides with similar Rf values both had N-terminal aspartic acid. Hence the above L-aspartic acid in mycobactin must occupy position 5 in the linear structure of the molecule shown in Fig. 1<sup>2</sup>.

It is now generally accepted that the predominant chemical bond in proteins is the amide or peptide linkage. Studies of amino acids occurring in tripeptides and peptide antibiotics indicate that some of these have peptide linkages involving the side chains of amino acids. Mycobactin contains seven residues of two dicarboxylic amino acids, aspartic and glutamic acid, in the molecule. Potentiometric titration and determination of free carboxyl groups indicate that the antibiotic lacks amino group but contains two free carboxyl groups indicating the presence of two side-chain peptide linkages which may be either  $\delta$ - or  $\gamma$ - or both. Hypobromite oxidation of the intact molecule also indicates the presence of at least one  $\gamma$ -peptide linkage. Further confirmation of  $\gamma$ - or other types of peptide linkage or quantitation of the number of  $\gamma$ -peptide linkages of the molecule were done by hydrolysis as well as by reduction of esterified mycobactin followed by hydrolysis and decarboxation. All these methods conclusively proved that all aspartic acid residues are L-linked and glutamic acids linked in mycobactin. Mycobactin does not react with hydroxylamine to give any hydroxamate indicating the absence of anhydride, lactone and ester linkages in the molecule. This is also confirmed by IR spectroscopy and titration of the molecule. The structure of the molecule so far elucidated is shown in Fig. 1.

## Biosynthesis

Studies on biosynthesis of mycobactin was undertaken in 1961 when a well knit hypothesis to account for the formation of protein has already been formulated and that of small peptides being synthesized by a template mechanism was just being confirmed. But less information was available at that time regarding the biosynthesis of a polypeptide, except the fact that RNA template synthesis could not be the case of mycobactin<sup>10</sup>. In this context the biosynthesis of a polypeptide of size of mycobactin (which is about one fourth the molecular weight of a protein) at the step sequence. Unless a mechanism of a well established nature for protein synthesis inhibits both growth and mycobactin formation and is not of a type which can be interpreted, but when nucleic acids have an effect they were said to affect the rate of growth of stationary culture growth RNA and DNA were not stop whereas mycobactin production continues at usual rate suggesting that RNA of high molecular weight is not involved in the formation of a chain. A change in the concentration of labeled amino acid in metabolic pools leads to a corresponding change in the specific activity of amino acid in the different peptide sequences of mycobactin molecule suggesting that mycobactin synthesis probably occurs by linear addition of amino acids.

A streptomycin dependent variant was isolated from the non-producer strain by single step mutant process. Strain was named the growth of the mutant (which may exceed growth of parent) but it was observed that when this mutant had been grown in presence of streptomycin but subsequently deprived of it mycobactin production remains quite normal, but protein synthesis is seriously impaired. This non-involvement of streptomycin dependency may therefore be taken as a further evidence in support of the previous conclusion that ribosome does not take part in mycobactin biosynthesis<sup>11</sup>.

The constituent D amino acids of mycobactin do not inhibit its synthesis in the whole cell fermentation. Studies with different non-producer mutants obtained by ultraviolet irradiation of the parent strain showed that none of the possible combinations between two mutant strains produces mycobactin.

The cell pool of the producer organism was found to contain four nucleotide phosphates. Uracil was present in all four nucleotide phosphates whose acid composition resembles that of mycobactin suggesting a probable relation between the two<sup>12</sup>.

An effective cell-free system prepared by postmortem assay of the producer strain was developed. The incorporation of labeled amino acids into mycobactin by the system is energy dependent and insensitive to RNAse action<sup>13</sup>.



leading to the formation of cyclic peptides like gramicidine S or tyrocidine<sup>11</sup>. Now in the light of these observations on L-proline dependent ATP-P<sub>i</sub> exchange and its sequential stimulation, we have studied the condition of inhibition of stimulations, caused by mycobacillin amino acids on ATP-P<sub>i</sub> exchange, initiated by L-proline and also the fate of mycobacillin synthesis by using sequential amino acid deprivation technique starting from L-proline.

In a cell-free system<sup>12</sup> from *Bacillus subtilis* B<sub>1</sub>, ATP-P<sub>i</sub> exchange was catalysed by L-proline at a pH optimum of 7.2. Further stimulation by component amino acids of mycobacillin was inhibited by deprivation from the synthesising system of even a single amino acid occurring at any point of the cyclic peptide. This inhibition however decreased with distance in the molecule of the given amino acid from L-proline. Peptides containing respectively two, three, four, five and six amino acids were isolated from the mycobacillin synthesising system by amino acid deprivation technique. The amino acid composition of these peptides, their C- and N-terminal amino acid residues and also stereo-configuration<sup>13</sup> were the same as those of peptides that would be obtained if mycobacillin synthesis occurred starting from L-proline and was interrupted at various points along the polypeptide chain. How the sequence of the polypeptide in absence of ribosomal synthesis is determined remains still a mystery.

#### SIGNIFICANCE OF ANTIBIOTIC SYNTHESIS

It is very relevant to ask the significance of antibiotic on the life process of a producer antagonist. How does an antibiotic affect a producer? Reports on the effect of antibiotic on producer organisms are very few. Growth inhibition at concentrations much higher than those required for sensitive organisms has been reported from various laboratories although the protoplasts of producer and sensitive organisms respond equally to the action of some antibiotics. Some times low yielding strains are more sensitive than high yielding ones as in the case of *S. griseus* towards streptomycin<sup>14</sup>.

It is observed that growth of producer *B. subtilis* B<sub>1</sub> is not supported in a synthetic medium when mycobacillin is incorporated as a nitrogen source. The antibiotic induces a lag in the growth of a producer or a nonproducer strain of *B. subtilis*. The producer organism also responds, to some extent, to mycobacillin with respect to its sensitive reactions like release of UV-absorbing materials, loss of gram positive character, agglutination, etc. Cells of *B. subtilis* B<sub>1</sub> in early log phase of growth are more sensitive to mycobacillin than those in late log phase or post log phase of growth while spores are completely insensitive to it. Though whole cells of the producer or a nonproducer strain of *B. subtilis* show little release of UV-absorbing materials on exposure to myco-

bacillin, protoplasts of both the strains do so to a considerable extent. The permeability of cells of *B. subtilis* is altered to the effect that retentivity of dyes like methylene blue is reduced. Thus the difference regarding the response of the producer and the sensitive organisms towards mycobacillin appears to be quantitative rather than qualitative<sup>27</sup>.

Significance was then sought for in terms of basic cellular metabolism. Most of the bacteria that produce peptide antibiotics are aerobic spore-former. The interrelation between the two processes was suggested by different laboratories. Bernlohr and Novelli<sup>18</sup> reported that the polypeptide antibiotic bacitracin is incorporated intact into the spore coat. Conflicting reports are however available. Hence it was considered worth while to reappraise the whole issue using *B. subtilis* which produces mycobacillin, an antibiotic peptide of almost the same size as bacitracin.

Both mycobacillin production and sporulation are initiated in the post log phase of growth of *B. subtilis* B<sub>1</sub>, reaching their maximum by about the same time. Mycobacillin is released during endotropic sporulation of vegetative cells, though not during germination of spore in complex growth medium<sup>11</sup>. Similar observations were also obtained by Bernlohr and Novelli<sup>18</sup>. Antisporogenic chemicals like glucose (in excess), diethylmalonate, acriflavin, fluorocetic acid, sodium bisulfite,  $\beta$ -phenethyl alcohol,  $\alpha$ -picolinic acid and m-tyrosine inhibit mycobacillin production.<sup>28</sup> Bernlohr Novelli<sup>18</sup>, and Paulus<sup>17</sup> also observed the inhibition of antibiotic synthesis by antisporogenic chemicals. By acriflavin and actinomycin D treatment, two types of mutants, oligosporous and asporogenous, were obtained. Mycobacillin production is affected adversely in oligosporous mutants whereas asporogenous mutants do not produce the antibiotic at all. Schaeffer<sup>19</sup> also isolated several Sp<sup>-</sup>Ab<sup>-</sup> mutants. Sporogenesis and antibiotic synthesis whose close association appears to be indicated by the inhibition of both the processes by common inhibitors and also by lack of antibiotic production by asporogenous mutants are however completely dissociated in Sp<sup>-</sup>Ab<sup>+</sup><sup>23,24</sup>, and in Sp<sup>+</sup>My<sup>-</sup> mutant in the authors' laboratory<sup>25</sup>.

During sporulation specific cell wall lytic enzymes<sup>26</sup> developed which cause considerable amount of lysis of cell walls and release of constituent peptides<sup>21</sup>. Now the amino acid composition of cell wall or spore coat of *B. subtilis* B<sub>1</sub> differs both qualitatively as well as quantitatively from that of mycobacillin. Therefore mycobacillin can not be assumed to be a structural component of cell wall or spore coat of the producer *B. subtilis* B<sub>1</sub><sup>29</sup>. The conclusion is consistent with that of Snake<sup>22</sup> and Brenner<sup>24</sup> with regard to bacitracin—and polymyxine B-producing *B. licheniformis* and *B. polymyxa* respectively.

Thus the close association as also dissociation between sporulation and antibiotic synthesis has yet to be assessed both genetically and biochemically.

#### MODE OF ACTION

The genus *Bacillus* elaborates a number of polypeptide antibiotics. These are generally antibacterial whereas mycobacillin, a polypeptide antibiotic also elaborated by the same genus, is exclusively antifungal. A good number of antifungal antibiotics are known but only a few of them except those of the polyene type have been investigated with regard to the mode of action. Different polyene antibiotics seem to act by altering cellular permeability<sup>10</sup>. Polymyxin a cyclic peptide not antifungal, but antibacterial is supposed to act on the osmotic barrier of sensitive cells<sup>11</sup>.

Mycobacillin is a broad spectrum antibiotic being active against skin pathogens, plant pathogens and also saprophytic fungi. Regarding its action on a sensitive strain of *Candida albicans*<sup>12</sup> it has been observed that it enhances lag period<sup>4</sup> but does not affect energy yielding processes like respiration and glycolysis. Though mycobacillin slightly affects the oxidation of its constituent amino acids, protein synthesis as measured in terms of incorporation of labelled leucine, serine or glycine into TCA precipitable fraction, remains unaltered in its presence. None of the constituent amino acids of mycobacillin can antagonize the growth inhibiting property of the antibiotic. It brings about agglutination of cells of a sensitive strain *Candida albicans*, changes its Gram-character and causes release of UV-absorbing materials from the cells.

Now detailed kinetic studies on mycobacillin sensitive reactions indicate that visible agglutination lags behind fall in viability which is also not quantitatively related to release. Thus none of the above mycobacillin-sensitive reactions alone can be considered as responsible for the antifungal action of the compound.

It is observed that sterols e.g. cholesterol, ergosterol, ergocalciferol and also lecithin can antagonise the growth inhibitory property as well as the leakage action of mycobacillin.<sup>13</sup> The action of polyene antibiotic has also been reported to be antagonised by sterols and lipids. Lipid like compounds were isolated from a mycobacillin sensitive organism *A. niger* and fractionated into neutral and phospholipid components which were found to contain mainly cholesterol and lecithin respectively. Both these lipid fractions isolated from the same very sensitive organism antagonise the growth inhibiting property of mycobacillin and may therefore be considered as the binding site for the compound. The antibiotic does not interfere with their biosynthesis. Sterols and lipid exert their antagonistic action, if added at 0 hr, subsequent addition fails to antagonise mycobacillin action. Electrophoretic, chromatographic

and spectrophotometric behaviour of mycobacillin, cholesterol and lipid either alone or in combination of their respective antagonising concentrations, indicates that antagonism might result from chemical interaction between the antibiotic and antagonist. Similar hypothesis has been advanced to account for the antifungal action of polyenes<sup>10</sup> which are chemically different from peptide. Studies with cholesterol derivatives and lecithin components indicate that 3-hydroxyl group must be free for cholesterol to act as an antagonist and that unsaturated oleic acid is the reactive component of lecithin<sup>11</sup>. There still remain the questions regarding the chemical nature of materials released by mycobacillin, nature of the reactive groups involved in lipid antibiotic antagonisation reaction and finally the subsequent effect of this very interaction on the dynamic structure and function of membrane.

#### EVALUATION OF MYCOBACILLIN OR ITS DERIVATIVES AS AN ANTIFUNGAL DRUG

Polypeptide antibiotics are generally toxic. Mycobacillin is greatly inactivated in presence of serum which limits its possible use as a drug. Reduction in hemolytic action of gramicidin has been variously reported. Acetylation lowers antifungal activity of mycobacillin, the inhibitory concentration (mg/ml) for the di- and tri-acetyl derivatives being 35-40 and 40-48 respectively as against 15-20 for mycobacillin; but acetylation gives complete protection against serum inactivation of the antibiotic whose inhibitory concentration is increased tenfold in its presence<sup>12</sup>. Esterification of mycobacillin with different alcohols decreases its antifungal activity as otherwise observed for esterified subtilin. However, esterification protects it partially from inactivation by serum. An ideal derivative has yet to be looked for.

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